56th Annual
Drosophila Research Conference

Full Abstracts

Note – Late Abstracts are at the end of the document

Sheraton Chicago Hotel & Towers
Chicago, IL
March 4-8, 2015

Sponsored by The Genetics Society of America
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Plenary Session 1

Cell division and epithelial tissue morphogenesis. Yohanns Bellaiche. Genetics and Developmental Biology Department, Institut Curie, Paris, France.

Questions related to embryo shape or morphogenesis have haunted developmental biologists for decades. Recent advances in imaging, cell biology, signal transduction and biophysics have framed the study of tissue morphogenesis in terms of collective cell dynamics and the interplay between biochemical and mechanical processes. Recent findings have confirmed that proliferative epithelial tissues reshape via morphogenetic processes such as cell shape change and cell rearrangements. Yet cell division remodels adherent junctions and modulates both tissue mechanics and tissue dynamics. Therefore its role and interplay with the other morphogenetic processes need to be understood to decipher the mechanisms of tissue morphogenesis. Moreover, given the large size of some proliferative tissues, challenging questions can be addressed: How do local and long-range mechanical effects contribute to tissue dynamics? How do the combinations of several signaling pathways or gene expression patterns specify distinct local cell dynamics leading to the emergence of several morphogenetic movements within a given tissue? During my talk I will describe some of our latest works that aim to understand the mechanisms of mitotic spindle orientation, propagation of cell polarization through division and how cell division impacts on the global shape and organization of epithelial tissue.

Plenary Session 1

Genetic conflicts during meiosis drive innovation in centromeric proteins. Harmit Malik. Dept Basic Sci & HHMI, Fred Hutchinson CA Res Ctr, Seattle, WA.

In female meiosis in both plants and animals, only one of four meiotic products is included in the oocyte. This creates a scenario in which chromosomal elements must compete during female meiosis for evolutionary success. By virtue of their important role in microtubule recruitment, centromeric regions are especially well poised to 'cheat' in female meiosis. While changes or expansions of centromric DNA can result in greater transmission through female meiosis, they can have deleterious consequences in male meiosis and even mitosis. Rapid alterations in centromeric DNA-binding proteins can negate the female meiotic advantage and restore male meiotic fitness. Thus, a Red Queen conflict plays out between centromeric DNA, on one hand and centromeric proteins, on the other, despite both playing essential roles in chromosome segregation. We study the important consequences of changes in essential centromeric proteins (e.g. Cid) as a result of these genetic conflicts. These antagonistic interactions can not only result in the recruitment of evolutionarily young genes (e.g. Umbrea) for essential centromeric function, but also the loss of ancestrally essential centromeric proteins that were rendered dispensable by changes in chromosome architecture. Such rapidly evolving centromeric protein-DNA interactions can also profoundly influence postzygotic isolation between incipient species.

Plenary Session 1

Coordinate Migration of Mesoderm Cells in the *Drosophila* Embryo. Angela Stathopoulos. Division of Biology and Bioengineering, Caltech, Pasadena, CA.

A common function of many genes expressed along the dorsal-ventral axis of *Drosophila* embryos is to coordinate the cell movements that encompass gastrulation. Therefore, a major focus of the lab is to analyze how these genes, signaling molecules and transcription factors, control mesoderm migration during gastrulation, the process which transforms the early embryo into a multilayered structure. With technical advances in live in vivo imaging and novel quantitative analyses, we have shown that mesoderm migration is a directed process; that cells move from ventral-most to dorsal-most regions of the ectoderm in a coordinate fashion. Our work has shown that FGF signaling is not absolutely required for movement, but instead that it supports collective movement of mesoderm cells. In the absence of FGF signaling, those cells in contact with the ectoderm are competent to migrate in a directional manner; while those that cannot contact the ectoderm exhibit random movements, and lose the ability to move directionally. Recently, we have found that FGF and particular cell adhesion mutants share phenotypes suggesting that FGF signaling modulates cell adhesion to support collective cell migration of the mesoderm. In addition, we have also developed caudal visceral mesoderm (CVM) migration as a novel system to study the coordinated movement of groups of cells. CVM migration is the longest migration in all of Drosophila embryogenesis and yet, surprisingly, little is known about this process, which is pivotal for proper gut development. Using live in vivo imaging and quantitative analyses, we are investigating how CVM cells, which move as a loosely-associated cluster of cells, coordinate their movement. FGF signaling also impacts migration of CVM cells supporting their directed migration as well as survival; yet, other signals surely contribute to effective CVM cell guidance, and several putative guidance mechanisms are currently being investigated. The coordinate migration of these two mesoderm cell populations will be compared and contrasted. FGF signaling impacts both cell movements but, presumably, has distinct roles. Interactions with other cell types also are important.

Plenary Session 1

Signaling in embryos: molecules, models, and movies. Stanislav Shvartsman. Lewis-Sigler Institute for Integrative Genomics, Princeton Univ, Princeton, NJ.

Transient activation of the highly conserved extracellular signal regulated kinase (ERK) establishes precise patterns of cell fates and morphologies in developing tissues. Quantitative parameters of these transients are essentially unknown, but a growing number of studies suggest that changes in these parameters can lead to a broad spectrum of developmental abnormalities. We provide a detailed quantitative picture of an ERK signaling event in the early Drosophila embryo, an experimental system that offers unique opportunities for highthroughput studies of developmental signaling. Our analysis reveals an ERK activation transient that is fully explained using a mechanistic model in which a temporal pulse in the production of a short-ranged ligand feeds into a simple signal interpretation

system. The quantitative data integration approach that led to this model, based on the information from fixed embryos and live imaging, can be extended to other developmental systems that rely on ERK activation. .

Plenary Session 1

Addressing complexity of Notch in cancer: When less is more. Maria Dominguez[1], Nahuel Villegas[1], Ede Migh[2], Vanina Da Ros[1], Diana Vallejo[1], Jesús García-Castillo[1], Irene Gutiérrez[1], Silvia Xargay[3], Dolors Colomer[3], Maria L. Toribio[4], József Mihály[2]. 1) Inst Neurociencias, UMH y, CSIC, Avd. Ramón y Cajal, s/n 03550 San Juan, Spain; 2) Biological Research Centre Hungarian Academy of Sciences 6726 Szeged, Temesvári krt. 62; 3) IDIBAPS-CEK Building (c/ Rosselló 149-153, Barcelona); 4) Centro de Biología Molecular "Severo Ochoa" Nicolás Cabrera 1 Universidad Autónoma de Madrid Campus de Cantoblanco 28049 Madrid.

Despite recent therapeutic advances, cancer still remains a pressing health concern. Cancer is a cellular phenomenon that occurs within the context of normal tissues and a normal microenvironment within the organism. As such, one of the major challenges is to understand how the initiating oncogenic lesions that confer malignant properties on cells are integrated with, and perturb, the physiological processes that regulate cell proliferation and growth, invasion, migration, and metabolism. Our work over these years has contributed to show how inappropriate Notch signalling instructs abnormal growth through partnership with other signalling pathways and factors. Attempts to target tumours generated by cooperation have repeatedly failed to stop cancer due to secondary oncogene addiction, as it has been shown for Notch- Pten/Akt cooperative tumours. Moreover, Notch signalling has key roles in maintenance of barrier function at epithelial organs like the gastrointestinal track and in the differentiation of functionally critical cell types. As such, indiscriminate abrogation of Notch poses a drug discovery challenge. In this talk, I will discuss data from complementary large-scale cancer-causing genes and a recent proteomic analysis complemented with a high-throughput compound screen applied to Notch-Pten tumours. These studies identify unforeseen targets of the cross-talk between these important 'oncogenic' pathways. Importantly, one of the compounds identified in flies was highly selective to kill human cancer cells carrying Notch activating mutations without affecting normal healthy cells, and with a relative low IC50. I will discuss opportunities and proposed strategies to target Notch signalling system in cancer.

Plenary Session 1

Using Drosophila neuroblasts as a model for stem cell biology and tumorigenesis. Juergen Knoblich. IMBA - Institute of Molecular Biotechnology, Vienna, Austria.

Stem cells are of crucial importance for tissue development, maintenance and regeneration in our body. They also play a critical role in tumor formation and growth. We are using Drosophila neuroblasts as a model system to understand, how stem cell proliferation is controlled and how defects in the control mechanisms can lead to tumor formation. Drosophila neuroblasts undergo repeated rounds of asymmetric cell division generating one neuroblast and one more differentiated cell. The asymmetric segregation of the cell fate determinants Numb, Prospero and Brat is responsible for establishing distinct fates in the two daughter cells. When any of those determinants is mutated, neuroblasts over proliferate leading to the formation of lethal transplantable brain tumors. We have used Drosophila to identify new tumor suppressors that have helped us to characterize the molecular mechanisms leading to stem cell immortalization in tumors at a level of detail currently not possible in vertebrate models.

Plenary Session II

How Zelda promotes enhancer activity during zygotic genome activation. C. Rushlow[1], Y. Sun[1], C-Y. Nien[1], SM. Foo[1], H-Y. Liu[1], K. Chen[2], J. Zeitlinger[2]. 1) Department of Biology, New York University, New York, NY; 2) Stowers Institute for Medical Research, Kansas City, MO.

The Drosophila embryo begins to activate its genes about one hour after fertilization. Recent studies have shown that the transcription factor Zelda plays a global role in this process. Zelda is uniformly distributed, and activates batteries of genes necessary for early developmental events. Zelda also works together with the graded morphogens, Bicoid and Dorsal, to ensure the correct temporal and spatial expression of their target genes, which is essential for proper pattern formation. Thus, Zelda is a major hub in the early gene network orchestrating the activity of the many different sub-networks. Since Zelda is present in nuclei before other transcription factors, it was proposed to play a pioneering role by binding its motifs in target enhancers and increasing chromatin accessibility for the patterning factors to bind. To test this hypothesis, we compared genome-wide Dorsal binding and nucleosome occupancy between wild-type and *zelda*- embryos. We found that in the absence of Zelda, Dorsal binding decreases at target enhancers and this is accompanied by an increase in nucleosome occupancy. Strikingly, Zelda-bound regions, which are enriched for early acting enhancers, harbor DNA sequences that favor nucleosome formation and are thus intrinsically “closed“. We propose that enhancers requiring precise spatial and temporal regulation have high intrinsic nucleosome occupancy, and that Zelda acts to lower the nucleosome barrier, thus facilitating competition between patterning factor binding and nucleosome occupancy. Our results represent a general model for enhancer function and a paradigm for mechanisms of genome activation in higher organisms.

Plenary Session II

Asymmetric stem cell division in Drosophila testis. Yukiko Yamashita[1,2]. 1) Ctr Stem Cell Biol, Univ Michigan, Ann Arbor, Ann Arbor, MI; 2) Howard Hughes Medical Institute.

Asymmetric stem cell division balances self-renewal and differentiation, contributing to tissue homeostasis. Drosophila male germline stem cells (GSCs) reside in a specialized microenvironment (the niche), wherein they divide asymmetrically by orienting their mitotic spindles with respect to the niche. Many cellular components, such as sister chromatids, centrosome and midbody, are inherited in a

non-random manner during asymmetric GSC divisions. Here we provide evidence that the stem cell niche actively regulates spindle orientation, independent of their role to provide self-renewing signals. We also show discuss the biological significance of numerous asymmetries observed during asymmetric GSC divisions.

Plenary Session II

Mechanisms and Functions of RNA Silencing Pathways. Phillip Zamore. RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA 01605.

Animals produce three distinct types of small silencing RNAs: small interfering RNAs (siRNAs), microRNAs (miRNAs), and PIWI-interacting RNAs (piRNAs). What unites these three pathways is the use of a small RNA to guide a member of the Argonaute protein family, an ancient class of nucleic acid-binding proteins found in all kingdoms of life. siRNAs play critical roles in anti-viral defense, particularly in invertebrates, whereas miRNAs regulate mRNA expression. Both siRNAs and miRNAs derive from double-stranded RNA precursors and are found in virtually all tissues. In contrast, piRNAs are largely restricted to germ cells and their supporting somatic cells; piRNAs derive from single-stranded transposon transcripts, long non-coding RNAs, and mRNAs. All three classes of animal small silencing RNAs act on RNA targets, either by b cleaving the targets or binding them stably. In contrast, eubacterial Argonaute proteins can use DNA or RNA guides and can act directly on DNA. The biogenesis of eubacterial guide DNAs and RNAs is not yet understood. Argonaute proteins imbue their guides with properties distinct from those intrinsic to nucleic acids. We seek to understand how Argonaute proteins alter the thermodynamic and kinetic properties of their RNA and DNA guides and how these fundamental biophysical changes reflect the in vivo functions of small silencing RNAs in both eukaryotes and prokaryotes. To this end, we combine biochemistry and genetics in insects, mice, and bacteria to analyze RNA silencing pathways in vitro and in vivo.

Plenary Session II

Control of Proliferative and Immune Homeostasis in the Aging Intestine. Heinrich Jasper. Buck Institute for Research on Aging, Novato, CA.

Proliferation of stem cells has to be carefully controlled to maintain long-term regenerative capacity of high-turnover tissues while preventing cancer. We study the *Drosophila* intestine as a genetically accessible model in which to explore stem cell function. Intestinal stem cells (ISCs) over-proliferate in aging flies, limiting lifespan. In recent work, we have explored some of the underlying causes of this hyper-proliferative phenotype, and have established a role for age-related immunosenescence and associated commensal dysbiosis in this breakdown of homeostasis. We are currently exploring the integration of oxidative stress and ER stress signaling in the control of ISC proliferation, as well as the interaction of ISCs with hemocytes in the regulation of tissue homeostasis. Our findings deepen our understanding of the regulation of proliferative homeostasis in aging barrier epithelia, and suggest potentially conserved mechanisms by which proliferative homeostasis can be preserved in the long term, extending lifespan. .

Plenary Session II

Decapentaplegic and the control of growth in the *Drosophila* wing imaginal disc. Matthew Gibson, Takuya Akiyama. Stowers Institute for Medical Research, Kansas City, MO 64110 USA.

As a central model for morphogen action during animal development, the Bone Morphogenetic Protein 2/4 (BMP2/4)-like ligand Decapentaplegic (DPP) is proposed to direct both growth and pattern formation in the developing *Drosophila* wing imaginal disc. While the patterning role of DPP secreted from a stripe of cells along the anterior-posterior compartmental boundary is well-established, the mechanism by which the DPP gradient directs cell proliferation remains controversial and poorly understood. Here, to determine the precise spatio-temporal requirements for *dpp* in wing development, we utilize CRISPR/Cas9-mediated genome editing to generate FRT-dependent conditional null alleles. By removing the *dpp* locus from cells in the stripe domain with *dpp-Gal4*-driven *flipase*, we confirm the expected requirements for the *dpp* gradient in activation of a downstream phospho-mad gradient and regulation of the transcriptional targets *spalt*, *optomotor-blind* and *brinker*. Surprisingly, however, third instar wing discs devoid of compartmental *dpp* expression maintained relatively normal cell proliferation and exhibited only mild defects in growth. In this presentation, I will describe these and additional results which are consistent with a revised temporal model for the role of *dpp* in imaginal disc growth.

Plenary Session II

Flies and Alcohol: Interplay of Nature and Nurture. Ulrike Heberlein[1], Ulrike Heberlein, past and current lab members[2]. 1) HHMI/Janelia Farm Research Campus, Ashburn, VA; 2) University of California at San Francisco, San Francisco, CA.

Alcohol is the most widely used and abused drug in the world with devastating medical and social consequences. The challenges associated with human studies have led to the development of animal models to investigate the molecular and neural mechanisms underlying both the acute and chronic effects of alcohol consumption. Although rodent models have been used widely and for a very long time,*Drosophila melanogaster* has emerged as a relevant model organism in the past two decades. During acute exposure to moderate concentrations of ethanol, adult flies increase locomotor activity; higher alcohol concentrations induce motor incoordination and sedation. Chronic ethanol exposure results in tolerance, dependence and relapse-like behavior. Flies also exhibit more complex addiction-like behaviors, including a lasting attraction for a cue that predicts ethanol intoxication and a preference for consuming ethanol-containing food, even if made unpalatable. Furthermore, social experiences, such as isolation and sexual rejection also affect alcohol-related behaviors in Drosophila. Importantly, many of the molecular, cellular and neurobiological processes that govern this variety of alcohol-related behaviors appear to be conserved in flies and mammals.

I will discuss the validity of Drosophila as a model for understanding alcoholism, provide a historical perspective, and present a synopsis of our current work.

1

A quantitative study of the *de novo* nucleolus formation in *Drosophila melanogaster* embryos. Hanieh Falahati[1,2], Barbra Pelham-Webb[2], Shelby Blythe[2], Eric Wieschaus[2]. 1) Lewis-Sigler Institute for Integrative Genomics; 2) Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton NJ. 08544.

The nucleolus is one of the largest eukaryotic compartments not bound by membranes. Mature oocytes lack this organelle and the nucleolus assembles during early embryogenesis in a process known as*de novo* nucleologenesis. Although nucleologenesis has been studied in several organisms, it is not well understood in *D. melanogaster*. In this study, we investigate the underlying mechanisms of the *de novo* nucleus formation in *D. melanogaster* via time-lapse confocal imaging of several nucleolar proteins, quantitative image analysis, and genetic approaches. Our results indicate that the nucleolus forms at nuclear cycle 13 in *D. melanogaster*, and is preceded by transcription of rDNA in earlier nuclear cycles. Also we have developed a novel approach that enables us to compare the timing of nucleolus formation with the time when nucleolar proteins begin to separate out as local non-homogeneities inside the nucleus. Using this approach we show that, unlike other organisms, the studied proteins do not localize to the nucleolus precursor bodies (NPBs) prior to the nucleolus formation. Employing the existing genetic tools in this simple system, we show that some of the nucleolar proteins can assemble to sub-nuclear compartments even in the absence of rDNA and rRNA. Finally, we use different quantitative and genetic approaches to study the factors that affect the timing of the nucleologenesis in *D. melanogaster*.

2

Examining chromatin structure in different states of G_0. Yiqin Ma, Laura Buttitta. MCDB, University of Michigan, Ann Arbor, MI.

States of cellular withdrawal from the cell cycle or G_0 can range from readily reversible to permanently postmitotic. We are interested in how different states of G_0 are controlled during development and why some are more reversible than others. Emerging evidence suggests a close relationship between a repressive chromatin structure and the silencing of cell cycle genes during the postmitotic state, but whether there are differences in the chromatin state between reversible and permanent cell cycle exit remain unclear. We focused our studies on the *Drosophila* pupal wing at a stage where the cells transition from active proliferation to a postmitotic state. We find there are two stages of G_0 in this tissue, a flexible G_0 period where cells can be induced to re-enter the cell cycle under specific genetic manipulations and a state we call "robust", where cells become strongly refractory to cell cycle re-entry. Compromising the flexible G_0 by driving ectopic expression of cell cycle activators causes a global disruption of heterochromatin-associated histone modifications such as H3K27 trimethylation and H3K9 trimethylation, as well as their associated repressors, Polycomb and heterochromatin protein 1(HP1). However, this disruption of heterochromatin is reversible. When cells enter a robust G_0 state, even in the presence of ectopic cell cycle activity, hereochromatin associated modifications are restored with a notable increase in HP1, suggestive of a state similar to oncogene induced senescence. However, compromising the H3K27 methyltransferase *Enhancer of zeste*, and/or HP1 cannot prevent the robust cell cycle exit, even in the face of normally oncogenic cell cycle activities. This suggests that additional chromatin modifiers must act to promote a repressive chromatin state during robust G_0. We are therefore taking an unbiased approach to examine and compare the chromatin structure in wings during proliferation, flexible G0 and robust G0, to understand how these states differ in vivo.

3

Chromatin Modifier Trithorax Regulates Systemic Signaling during *Drosophila* Imaginal Disc Regeneration. Andrea Skinner, Sumbul Jawed Khan, Rachel K. Smith-Bolton. Cell & Developmental Biology, University of Illinois Urbana-Champaign, Urbana, IL.

Imaginal disc regeneration is an important response to damage and injury that involves both local regrowth of the lost tissue, as well as a systemic response through the *Drosophila* insulin-like peptide 8 (Dilp8) that delays entry to metamorphosis in order to give the larva time to regenerate. Through the use of a genetically induced ablation system, we are able to temporally and spatially control tissue ablation in larval imaginal wing discs, and thereby screen for genes that affect regeneration. Through this unbiased genetic screen we identified the chromatin modifier *trithorax* as necessary for regeneration. Animals with reduced levels of Trithorax are unable to delay entry into pupariation and metamorphosis long enough for completion of regeneration. This failure of damage-induced systemic signaling is in part due to abnormally high expression levels of the phosphatase *puckered,* which negatively regulates Jun N-terminal Kinase (JNK) signaling. The reduced levels of JNK activity then lead to decreased expression of*dilp8*, which is critical for the systemic response to tissue damage. Thus, using a genetic approach we have identified one specific way in which a chromatin modifier can regulate the response to tissue damage and enable regeneration. Given the dramatic changes in gene expression that occur throughout regeneration, future work will use genomic approaches to identify any broader roles that chromatin modification plays in the regulation of this process.

4

Growth coordination during regeneration occurs through nitric oxide regulation of steroid hormone production. Jacob Jaszczak, Jacob Wolpe, Anh Dao, Adrian Halme. Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA.

The proportional growth of distinct organs is not easily explained solely through tissue-autonomous or systemic mechanisms of regulation. Experiments in many different systems suggest that inter-organ communication coordinates growth between different tissues. In *Drosophila* larvae, imaginal disc damage activates a regenerative checkpoint that both extends the regenerative period of

development and slows the growth of undamaged imaginal discs. This growth reduction may function to coordinate regenerative growth with the growth of undamaged tissues. Dilp8 has been identified as an important regulator of the regenerative checkpoint and growth coordination. However, how Dilp8 mediates growth control has been unknown.
Nitric oxide synthase (NOS) has been shown to regulate developmental growth of imaginal discs through a previously uncharacterized mechanism. We find that NOS is necessary for Dilp8-dependent growth inhibition. We also find that nitric oxide production increases in the prothoracic gland (PG) during the regenerative checkpoint and during Dilp8 expression. Further, we show that the slowed growth is due to reduced ecdysone signaling during the regenerative checkpoint. In contrast to previously published experiments showing that NOS promotes ecdysone synthesis in post-feeding larvae, we find that NOS activity in feeding larvae reduces ecdysone synthesis. Based on these data, we propose that Dilp8 produced in regenerating tissues signals through NOS in the PG to decrease ecdysone signaling, thus coordinating regenerative growth with developmental growth. Surprisingly, we find that NOS activity in the PG does not regulate the developmental delay of the regenerative checkpoint. These data suggest that Dilp8 may signal directly to the PG to regulate growth and elsewhere to regulate developmental timing. Consistent with this hypothesis, we have identified a putative Dilp8 receptor required in the PG to specifically regulate growth during regenerative checkpoint activation and are currently characterizing this receptor's activity and association with Dilp8.

5

Both damage-responsive WNT expression and an age-dependent decline in regenerative capacity are mediated by a bipartite regulatory element in the WNT cluster. Robin Harris, Joshua Saul, Iswar Hariharan. MCB, University of California, Berkeley, Berkeley, CA.

For many organisms, the ability to regenerate damaged tissues diminishes with increasing maturity. The mechanisms responsible are not known, but their identification will be essential for the design of strategies to promote regeneration in adult organisms. *Drosophila* imaginal discs, when damaged *in situ*, are able to regenerate, but this ability diminishes dramatically during the third larval instar. This decline in regenerative capacity correlates with a reduction in the ability to upregulate *wingless* (*wg, Drosophila WNT1*) expression. Since WNT proteins are upregulated following tissue damage in diverse taxa, the mechanisms that regulate WNT expression following tissue damage are likely to be evolutionarily ancient.
To understand how damage-induced *wg* expression is activated, we have characterized an enhancer located in the WNT cluster that regulates the expression of two flanking WNT genes; *wg* and *WNT6*, during regeneration. Deletion of this enhancer permits normal development, but compromises regeneration. Dissection of the region reveals a bipartite structure that includes a damage-responsive module and a silencing element. The damage responsive module is robustly activated following various damaging stimuli, and importantly its activity is undiminished with age. In contrast, the silencer element has no enhancer activity on its own, but can attenuate expression mediated by the damage-responsive module in *cis*, and is responsible for the age-related decline of expression in older larvae. Thus the signaling mechanisms that mediate damage-responsive WNT expression are intact in older larvae, but are actively repressed by a silencing mechanism. We have used reporter genes and genome editing to identify key inputs to the damage-responsive module and the silencing element. Finally, our data show that targeting *Myc* expression to regenerating wound periphery cells using the damage-responsive module of the WNT enhancer can improve regenerative capacity, even in mature larvae, thus demonstrating a method to overcome an age-dependent decline in regenerative capacity.

6

Identification of the Dilp8 receptor and characterization of its role in the coordination of organs growth and developmental transitions. Julien Colombani, Ditte S Andersen, Pierre Leopold. Institut of Biology Valrose (iBV), CNRS UMR7277 / INSERM UMR1091 / UNS, Nice, France.

An important aspect of final size determination is the coordination of growth arrest among organs and its coupling with the program of development. We previously identified Dilp8 as a secreted peptide produced by growing and regenerating tissues that couples tissue growth and patterning with the developmental timing by acting remotely on the brain/ring gland complex to regulate ecdysone production (*Colombani et al. Science 336 - 582-5, 2012*). To better understand the mode of action of this new hormone, we designed a genetic screen aimed at identifying the receptor for Dilp8. For this, a developmental delay was induced by driving Dilp8 expression in the imaginal discs using the lexA/lexOp expression system. Candidate RNAi lines were then expressed under the control of different brain-specific drivers using the Gal4/UAS system and tested for their ability to rescue the pupariation delay induced by disc-derived Dilp8. To specifically screen for molecular components implicated in the reception of the Dilp8 signal, we used a subset of the TRIP and VDRC collections of transgenic RNAi lines targeting genes predicted to encode membrane-associated proteins, corresponding to approximately 1200 RNAi lines. This way, we identified a transmembrane molecule functionally required for Dilp8 action. Its down-regulation using brain drivers fully rescues the developmental delay induced by Dilp8 overexpression. The molecular characterisation of this novel receptor and its physiological implication in growth coordination mechanisms will be presented.

7

Identification of the ligand-receptor system that governs tumor-suppressive cell competition. Tatsushi Igaki[1,2], Masatoshi Yamamoto[1], Kei Kunimasa[1], Shizue Ohsawa[1]. 1) Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Kyoto, Japan; 2) PRESTO, Japan Science and Technology Agency.

Cell-cell interactions play an important role in maintaining tissue homeostasis including the removal of suboptimal or dangerous cells from the cell population. In *Drosophila* imaginal epithelia, clones of cells mutant for neoplastic tumor-suppressor genes such

as *scribble* (*scrib*) or *discs large* (*dlg*) are eliminated from the tissue by cell competition. We have previously shown that Eiger/TNF-JNK signaling plays a role in driving this tumor-suppressive cell competition. However, the initial events occurring at the interface between two cell populations to trigger the cell-elimination have been missing. To address this, we conducted an EMS-based genetic screen for genes required for wild-type "winners" to eliminate neighboring polarity-deficient "losers". We isolated a mutant *eld-4* (*elimination-defective-4*), which encodes a transmembrane protein that acts as a cell-surface ligand. Furthermore, we identified through an RNAi screen a receptor for Eld-4 (Eld-4R), which is required for polarity-deficient "losers" to be eliminated. We found that both Eld-4 and Eld-4R are accumulated at the interface between "winners" and "losers". The mechanism by which the Eld-4/Eld-4R system governs tumor-suppressive cell competition will be discussed.

8

Localization of Warts activation in vivo. Shuguo Sun[1], Venu Reddy[2], Ken Irvine[1]. 1) Waksman Institute, piscataway, NJ; 2) Tata Memorial Center, Navi Mumbai, Maharashtra, India.

Hippo signaling is a crucial pathway controlling organ growth and cell fate, which acts by regulating the activity of the kinase Warts. Multiple Hippo pathway components localize to apical junctions in epithelial cells, but the spatial and functional relationships among junctional components have not been clarified, nor is it known where within the cell Warts activation occurs. We report here that Hippo pathway components in the *Drosophila* wing imaginal disc are organized into distinct junctional complexes, including separate distributions for Salvador, Expanded, Warts, and Hippo. These complexes are reorganized upon Hippo pathway activation and increased Warts expression, when Warts shifts from associating with the Warts-inhibitor Jub to the Warts-activator Expanded. Using a phospho-specific antisera, we identify where Warts activation occurs in vivo: activated-Warts is found at apical junctions where Expanded, Salvador, Hippo and Warts overlap. Our observations define spatial relationships amongst Hippo signaling components in vivo and establish the functional importance of this localization to Warts activation..

9

Dscam Switches Slit Repulsion to Attraction via the Robo Receptor. Maryam Alavi, Minmin Song, Gracie Andrews, Taylor Gillis, Thomas Kidd. Dept. of Biology/MS 314, University of Nevada Reno, Reno, NV.

The navigation of axons to reach their correct targets is a critical part of neural development. Axons navigate via different axon guidance cues, including diffusible ligands such as Netrins and Slits. The receptor repertoire present, in the axonal growth cone, determines whether a cue elicits attractive or repulsive responses. Slit repels axons from the CNS midline by binding to the Robo (Roundabout) receptor. Netrin acts as a midline attractant through fra/DCC/Unc-40 and Dscam (Down syndrome cell adhesion molecule) receptors. We sought to demonstrate that Slit can also act as a midline attractant, via the Dscam receptor. *In vivo*, Slit is proteolytically cleaved into two fragments, Slit-N and Slit-C. Slit-N contains the Robo binding site and can also bind Dscam, whereas full length Slit (Slit-FL) can only bind Robo. We have mapped the binding sites within the Slit-N and Dscam molecules via cell culture to demonstrate that the interaction is strong and highly specific, suggesting that it is biologically relevant. Embryonic expression of transgenic Slit derivatives in various tissues suggests that Slit-N can simultaneously act as an attractant and as a repellent. However, Slit lacking the Robo binding site had no activity, despite the presence of the Dscam binding site. This suggested that Robo is required for the attractive function of Slit-N. We have found that Dscam and Robo preferentially form a complex in the presence of Slit-N, whereas Robo alone predominantly binds Slit-FL. We are using an *in vivo* assay system to prove this model, by varying levels of the receptors in the presence of ectopically expressed Slit-N. Our results suggest that Slit-N is one of the unidentified CNS midline attractants and explain why *robo* genes are exquisitely sensitive to gene dosage. The ability of Dscam, to modulate Robo output, explains why an extra copy of Dscam in trisomy-21 underlies some of the symptoms of Down Syndrome. Finally, the observation of opposing outputs, depending on relative receptor levels, can explain how a relatively small number of ligands give rise to the complexity of the nervous system.

10

***Drosophila S6 kinase like* inhibits neuromuscular junction growth by downregulating the BMP receptor Thickveins. Guoli Zhao**[1], Yingga Wu[1], Li Du[2], Qifu Wang[1], Wenhua Li[1], Yongqing Zhang[1]. 1) institute of genetics and developmental biology, CAS, Beijing, China; 2) College of Life Science, Hubei University, Wuhan, Hubei, China.

Synaptic connections must be precisely controlled to ensure proper neural circuit formation. In *Drosophila melanogaster*, bone morphogenetic protein (BMP) promotes growth of the neuromuscular junction (NMJ) by binding and activating the BMP ligand receptors wishful thinking (Wit) and thickveins (Tkv) expressed in motor neurons. We report here that an evolutionally conserved, previously uncharacterized member of the S6 kinase (S6K) family S6K like (S6KL) acts as a negative regulator of BMP signaling. *S6KL* null mutants were viable and fertile but exhibited more satellite boutons, fewer and larger synaptic vesicles, larger spontaneous miniature excitatory junctional potential (mEJP) amplitudes, and reduced synaptic endocytosis at the NMJ terminals. Reducing the gene dose by half of *tkv* in*S6KL* mutant background reversed the NMJ overgrowth phenotype. The NMJ phenotypes of *S6KL* mutants were accompanied by an elevated level of Tkv protein and phosphorylated Mad, an effector of the BMP signaling pathway, in the nervous system. In addition, Tkv physically interacted with S6KL in cultured S2 cells. Furthermore, knockdown of S6KL enhanced Tkv expression, while S6KL overexpression downregulated Tkv in cultured S2 cells. This latter effect was blocked by the proteasome inhibitor MG132. Our results together demonstrate for the first time that S6KL regulates synaptic development by facilitating degradation of the BMP receptor Tkv.

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Hippo and its negative regulator Strip regulate synapse formation at *Drosophila* larval neuromuscular junctions. Takahiro Chihara[1,2], Chisako Sakuma[1], Yoshie Saito[1], Tomoki Umehara[1], Keisuke Kamimura[3], Timothy Mosca[4], Nobuaki Maeda[3], Masayuki Miura[1,2]. 1) Dept Gen, Grad Sch Pharm, Univ Tokyo, Tokyo, Tokyo, Japan; 2) CREST, JST, Japan; 3) Dept of Brain Dev and Neural Reg, Tokyo Metro Inst of Med Sci, Japan; 4) Dept of Biol, HHMI, Stanford Univ, Stanford, CA.

The Hippo (Hpo) pathway is well known for its role in growth control in both flies and mammals. Although postmitotic roles of Hpo have also been uncovered such as regulation of dendrite tiling and photoreceptor specification, there has been no report of Hpo function in synapse development. Here, we show the role of Hpo and its negative regulator Strip in synapse formation of *Drosophila* larval neuromuscular junction (NMJ). We previously reported that Strip, an evolutionarily conserved protein functions as a platform for endosome maturation that is required for axon elongation (*Nat Commun* 5, 5180, 2014). In addition, we found that endogenous Strip is predominantly localized at presynaptic sites of NMJ. *strip* knockdown in motor neurons resulted in the significant increase in the number of small synaptic bouton called "satellite" bouton. Furthermore, *strip* knockdown larvae exhibited the defects in synapse transmission. As Strip was also reported as a component of STRIPAK (PP2A) complex, a negative regulator of Hpo in growth control, we examined the relationship between Strip and Hpo. First, we found that *strip* knockdown in S2 cells significantly increased the phosphorylation level of Hpo. Consistent with this, the satellite bouton phenotype by *strip* knockdown was significantly suppressed in *hpo* heterozygous background, suggesting that Strip negatively regulates Hpo activity in synapse development. We also observed overexpression of Hpo caused the satellite bouton phenotype, suggesting that Hpo positively regulates satellite bouton formation. Here we would like to present the molecular mechanism how Hpo and Strip regulate synapse development. .

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Tenectin (Tnc) recruits Integrins to stabilize postsynaptic structures at the *Drosophila* NMJ. Qi Wang, TaeHee Han, Mihaela Serpe. NICHD/NIH, Bethesda, MD.

Synapses are characterized with the specific presynaptic, perisynaptic and postsynaptic domains. Those morphological and functional different structures are responsible for the synapse formation and development. However, how synapses build up the precise boundaries to organize those dynamic structures and regulate their cellular interactions is largely unknown.We have recently discovered that Neto (Neuropilin and Tolloid-like) is an essential auxiliary protein required for the recruitment and stabilization of ionotropic glutamate receptor (iGluRs) at the *Drosophila* NMJ. Neto also regulates the proper recruitment of postsynaptic components and organizes the synaptic structures. How Neto performs all these functions during synaptogenesis remains to be understood. To address this question, we carried out a genetic screen to isolate new *neto*-interacting candidates involved in synapse assembly and development.Here we report that Tenectin (Tnc), a large mucin secreted from the mortor neurons, ensures bouton integrity at the NMJ. *tnc* maps to overlapping deletions that interact genetically with *neto*. *tnc* mutants are partial lethal; the adult escapers have severe locomotor defects and the mutant larvae have altered NMJ physiology, i.e. low mini frequency. Tnc-deprived NMJs have normal iGluRs synaptic clusters, and electron micrographs show normal synaptic structures, but the synaptic boutons are collapsed. *tnc* mutants have impaired synaptic accumulation of βPS integrin and recapitulate some of the *βPS* mutant phenotypes. Expression of Tnc in motor neurons, but not in the muscle, can rescue the synaptic distribution of βPS integrin and partially restore the defects of *tnc* mutants. Take together our data indicate that Tnc, secreted from the motor neurons, functions as a ligand for integrins in the synaptic cleft. Tnc anchors βPS at perisynaptic locations, where integrins act to maintain the bouton shape and ensure proper development of synaptic structures.

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Specification of individual adult motor neuron morphologies by combinatorial transcription factor codes. Jonathan Enriquez[1], Myungin Baek[2], Meredith Peterson[1], Lalanti Venkatasubramanian[1], Ulkar Aghayeva[1], Richard Mann[1]. 1) Department of Biochemistry and Molecular Biophysic, Columbia University Medical Center, New York, NY; 2) NYU Neuroscience Institute, Department of Neuroscience and Physiology, NYU School of Medicine.

How the highly stereotyped morphologies of individual neurons are genetically specified is not well understood. We identify five transcription factors (TFs) expressed in a combinatorial manner in seven post-mitotic adult leg motor neurons (MNs) that are derived from a single neuroblast in *Drosophila*. Unlike TFs expressed in mitotically active neuroblasts, these TFs do not regulate each other's expression. Removing the activity of a single TF resulted in specific morphological defects, including muscle targeting and dendritic arborization, and in a highly specific walking defect in adult flies. In contrast, when the expression of multiple TFs was modified nearly complete transformations in MN identities were generated. These results suggest that the morphological characteristics of a neuron are dictated by a combinatorial code of morphology TFs (mTFs). mTFs function at a previously unidentified regulatory tier downstream of factors acting in the NB, but independently of factors that act in terminally differentiated neurons.

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Molecular Dissection of a Cell Specification Regulatory Cascade. Johannes Stratmannn[1], Hugo Gabolindo[2], Jonathan Benito-Sipos[2], Stefan Thor[1]. 1) Department of Clinical and Experimental Medicine, Linköping University, SE-581 83 Linköping, SWEDEN; 2) Departamento de Biología, Universidad Autónoma de Madrid, Cantoblanco, E 28049 Madrid, SPAIN.

Specification of the myriad of unique neuronal sub-types found in the nervous system depends upon complex regulatory cascades. These involve spatial and temporal cues, as well as cell-type specific determinants, often acting in combinatorial codes to determine final cell fate. While some progress has been made with respect to the identification of several such genetic cascades, little is known

about the molecular flow from spatio-temporal cues to final cell fate. To address this issue we are using the Nplp1 neuropeptide cells in the ventral nerve cord; a subset of Apterous neurons, generated by thoracic neuroblast 5-6 (NB5-6T), as a model.Nplp1 cell fate specification involves a cascade of different transcription factors and co-factors (TF), which feed forward onto their specific downstream enhancer targets to ultimately specify the Nplp1 cell fate in a combinatorial fashion. These involve positional TFs (Hox and Hox co-factors), temporal and sub-temporal TFs (Castor, Sqz and Nab), acting on a set of postmitotic cell fate determinants (terminal selector genes), which in turn act at later stages to dictate the final cell identity. We have identified six enhancers involved in the Nplp1 specification cascade and we have begun dissecting their organization by extensive in vivo studies. These involve mutant and misexpression analysis, enhancer mutagenesis, and ChIP-seq of the identified TFs. In addition, the CRISPR/Cas9 system is being used to delete specific enhancer regions for each gene in the regulatory cascade.These studies reveal complex combinatorial molecular action of TFs to sequentially specify the unique Nplp1 cell fate, involving the integration of temporal and positional cues onto terminal selector genes in the NB5-6T neuroblast and post-mitotic neurons to ultimately determine final cell fate, evident by the final activation of the Nplp1 enhancer. To our knowledge, this study represents the first case where the molecular logic of spatial and temporal cues onto final and unique neuronal cell fate, within the detailed context of a well-mapped neuroblast lineage, has been resolved.

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Morphogenesis and development of the postembryonic cortex glial niche. Renee Read, Coston Rowe. Pharmacology, Emory University School of Medicine, Atlanta, GA.

Like many types of stem cells, neural stem cells reside within a specialized cellular microenvironment, the niche, which maintains stem cell self-renewal and promotes differentiation of daughter cells. In the *Drosophila* central brain, post-embryonic neuroblasts, which are multipoint stem-cell like neural progenitor cells, reside within a cortex glial niche that coordinates neuroblast proliferation, self-renewal, and differentiation. To understand the origins of the cortex glial niche and to identify the molecular pathways that govern niche-neural stem cell interactions, we have used a combination of cell-type specific genetic manipulation and lineage analysis. Our data show that cortex glia in the niche in the central brain arise in early neurogenesis and comprise a lineage of separately proliferating cells distinct from the neuroblasts, and that, throughout post-embryonic neural development, proliferation and morphological elaboration of niche cortex glia controls neurogenesis. Through genetic analyses, we discovered that the PDGF-PDGFR signaling axis, which controls the development of oligodendroglia in mammals, is required for proper development of the glial niche in the *Drosophila* central brain. Our data indicate that neuroblasts express PDGF ligands and that niche cortex glia require Pvr, a PDGFR-like receptor. Loss of Pvr signaling reduces proliferation of the niche cortex glia and impairs morphogenesis of the niche, which causes non-cell-autonomous defects in neuroblast maintenance. Moreover, gain of PDGFR signaling in the glial niche contributes to tumorigenesis in a *Drosophila* model of malignant glioma. Through our ongoing analyses, we have also identified signaling pathways that act in parallel to and downstream of PDGFR signaling in the niche cortex glia in normal development and neoplasia. Our data indicate a novel function of PDGFR signaling in the neurogenic niche, and, more importantly, demonstrate a remarkable evolutionary conservation of PDGFR signaling in glial development. Continued studies of the glial niche in *Drosophila* will lead to the identification of new factors and new mechanisms that control the development and function of neural stem cells, glial progenitor cells, and glioma cells.

16

A novel neurotropic pathway regulates core features of neuronal identity. Colleen McLaughlin, Heather Broihier. Case Western Reserve University, Cleveland, OH.

Mammalian neurotrophic pathways regulate an extraordinary range of cellular processes from neuronal survival and axon guidance early in development to morphological and functional plasticity at later stages. Though neurotrophin-mediated processes have been identified, questions remain regarding context-specific functions, mechanisms of signal transduction, and the role of transcriptional regulation in these molecular pathways. We have recently identified a novel neurotrophic pathway in Drosophila that regulates neuronal survival and plasticity, thus providing an outstanding opportunity to define neurotrophic activity in a highly tractable model system. Our lab has a longstanding interest in neuronal roles of the FOXO transcription factor. In a genetic screen, we identified the neurotrophin receptor Toll-6 as a putative upstream regulator of FOXO. Both FOXO and Toll-6 are neruonally expressed and exhibit neuroprotective activity in the embryonic CNS. Toll-6 also regulates FOXO nucleo-cytoplasmic shuttling. Further supporting a Toll-6-FOXO pathway, Toll-6 modulates Akt kinase activity, a known regulator of FOXO. Additionally, FOXO and Toll-6 serve similar neuronal functions at the larval neuromuscular junction (NMJ). For example, motoneuronal overexpression of FOXO or Toll-6 drives substantial synapse overgrowth. Also, FOXO is required for Toll-6-dependent synaptic overgrowth, thus providing evidence for FOXO activity downstream of Toll-6. Furthering the phenotypic similarity, Toll-6 and FOXO single and double mutants exhibit synapse organization defects in the form of decreased active zones and enhanced microtubule stability at the NMJ. Using established plasticity assays, I find that Toll-6 and FOXO are essential for activity-dependent structural plasticity. Surprisingly, both active zone and plasticity defects in FOXO nulls are ameliorated by genetically reducing microtubule stability. This unexpected finding indicates that enhanced presynaptic microtubule stability can impair plasticity at the NMJ. Taken together, these data argue that a novel Toll-6-FOXO pathway has neurotrophic function in the Drosophila CNS.

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Molecular and cellular mechanism of epithelial invagination in the Drosophila salivary gland. Se-Yeon Chung, Sangjoon Kim, Deborah Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

During embryogenesis, the salivary gland (SG) primordia change geometry from two monolayered plates of tightly adherent epithelial

cells on the embryo surface into two elongated, fully internalized secretory tubes. Internalization of the SG absolutely depends on the FoxA transcription factor Fork Head (Fkh). Here, we reveal the mechanisms whereby SG cells internalize and we connect Fkh function to cytoskeletal changes driving this process. Using both live and fixed cell imaging, we show that internalization begins with formation of a unique rosette structure with a single central cell that initiates internalization by basal repositioning of its adherens junctions. Neighboring rosette cells subsequently internalize followed by cells in more ventral and anterior regions of primordium. We show that although apical constriction is observed during internalization, it is not required. We also demonstrate that loss of fkh affects only one of three types of myosin cables observed during internalization - dynamic apical trans-medial myosin cables. These myosin cables provide motive forces during internalization and deform the hexagonal packing of the primordia. Dynamic increases in Drosophila Rho-associated kinase (Drok) are observed in the apical domain of SG cells during internalization and Drok activity is required for membrane deformation and subsequent SG internalization. We also provide evidence that Fkh regulates transcription of folded gastrulation (fog), which encodes a secreted ligand that activates the signal transduction pathway controlling trans-medial myosin cable formation.

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Two Forkhead transcription factors regulate cardiac progenitor specification by controlling the expression of receptors of the fibroblast growth factor and Wnt signaling pathways. Shaad M. Ahmad[1], Pritha Bhattacharyya[1], Neal Jeffries[2], Stephen S. Gisselbrecht[3], Alan M. Michelson[1]. 1) Laboratory of Developmental Systems Biology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD; 2) Office of Biostatistics Research, National Heart, Lung and Blood Institute, NIH, Bethesda, MD; 3) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Cardiogenesis involves multiple biological processes acting in concert during development, a coordination achieved by the regulation of diverse cardiac genes by a finite set of transcription factors (TFs). Previous work from our laboratory identified the roles of two Forkhead TFs, Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) in governing cardiac progenitor cell divisions by regulating Polo kinase activity. These TFs were also implicated in the regulation of numerous other cardiac genes. Here we show that these two Forkhead TFs play an additional and mutually redundant role in specifying the cardiac mesoderm (CM): eliminating the functions of both *CHES-1-like* and *jumu* in the same embryo results in defective hearts with missing hemisegments. Our observations indicate that this process is mediated by the Forkhead TFs regulating the fibroblast growth factor receptor Heartless (Htl) and the Wnt receptor Frizzled (Fz), both previously known to function in cardiac progenitor specification: *CHES-1-like* and *jumu* exhibit synergistic genetic interactions with *htl* and *fz* in CM specification, thereby implying function through the same genetic pathways, and transcriptionally activate the expression of both receptor-encoding genes. Furthermore, ectopic overexpression of activated Htl in the mesoderm is able to partially rescue the defective CM specification phenotype seen in embryos doubly homozygous for mutations in *jumu* and *CHES-1-like*. Together, these data emphasize the robustness of the cardiac progenitor specification process mediated by Forkhead TFs regulating the expression of signaling pathway receptors, and illustrate the pleiotropic functions of this class of TFs in different aspects of cardiogenesis.

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The adult midgut progenitor cells are established through asymmetric cell division. Jessica R.K. Seifert[1,2], Ruth Lehmann[2]. 1) Farmingdale State College, Farmingdale, NY; 2) NYU School of Medicine, New York, NY.

The larval adult midgut progenitor cells (AMPs) have been shown to give rise to all cells of the adult midgut including the stem cells. The specification of the AMPs occurs in the embryo and is regulated by Notch signaling. Previous models have speculated that specification of the AMPs leads to delamination of these cells from the endodermal epithelium and our work showed that expressing an activated form of the Notch receptor leads to a loss of AMP specification and blocks delamination. Surprisingly, our current results indicate that AMP delamination is actually a result of asymmetric cell division. This asymmetric cell division results in the restriction of Escargot (Esg), an important stem cell factor in the adult intestine, to the AMPs. Both asymmetric division and Esg restriction appear to be dependent on Notch signaling. Additionally, we find that restriction of Esg occurs in both the anterior and posterior primordium of the endoderm, but the cell types generated from asymmetric division are not the same in both tissues. These results indicate that regional specification between the anterior and posterior endoderm occurs very early in embryonic development. Finally, we propose a revised model of AMP formation, which is based on the asymmetric cell division of endodermal cells.

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The Wnt signaling and cytoskeletal regulator APC2 controls stem cell niche size, architecture, and stem cell number in the Drosophila ovary. Stacie Oliver, Ezgi Kunttas-Tatli, David Vinson, Brooke McCartney. Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

The spatial arrangement of the stem cell niche and the cellular signaling that takes place there are precise and maintain stem cell self-renewal capability and multipotency. While signaling in the mature niche is well-studied, mechanisms governing niche establishment and architecture are not clearly understood. Here we report that the Wnt signaling and cytoskeletal regulator, Adenomatous polyposis coli (APC2) is required for proper niche size and architecture. Cap cells (CpC), a key component of the female germline stem cell niche, reside at the anterior tip of the germarium and are essential for maintaining stem cells in the niche. Loss of APC2 results in an increased number of CpC, their displacement away from the tip, and an increased number of germline stem cells (GSC). APC2 loss in somatic cells only of the germarium reproduced this phenotype, suggesting that increased GSC results from the increase in CpC. A separation of function allele of APC2 revealed that increased CpC is due to activation of Wnt, while CpC position requires the actin regulation function

of APC2. If inappropriate activation of Wnt in somatic cells increases CpC, we predict this may be at the expense of another somatic cell type. To test this hypothesis, we are examining the somatic cells in wild type and *APC2* null larval ovaries. Because somatic terminal filament cells (TF) and intermingled cells (IC), including escort cell precursors are adjacent to the CpC, they may contribute to CpC increase. Preliminarily we find that while TF are unchanged, IC inappropriately activate the Wnt target *engrailed*. We are currently testing whether IC contribute to the expanded niche. We also find that proper CpC position is necessary to regulate niche size and GSC number. Our preliminary results suggest that APC2 controls CpC position by regulating cortical actin with the formin Diaphanous. Consistent with this model, selective reduction of the actin cross-linkers Spectrin and Kelch in CpC results in CpC displacement. Taken together, our data suggest that APC2 regulates niche size and architecture through two distinct mechanisms.

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Investigating the role of pHi in promoting stem cell differentiation. Bryne Ulmschneider[1], Bree Grillo-Hill[2], Diane Barber[2], Todd Nystul[1]. 1) Anatomy, University of California, SF, San Francisco, CA; 2) Cell and Tissue Biology, University of California, SF, San Francisco, CA.

Throughout all stages of life, cells undergo regulated transitions from one identity to another. This fundamental process of development requires coordinated changes in the activity of diverse proteins within the cell. Decades of research have elucidated many signal transduction events and gene pathways critical for coordinating cell identity changes. However, changes in cell physiology and metabolism also coincide with developmental progression, but much less is known about whether or how these changes contribute to changes in cell identity. We found that pHi increases by about 0.4 pH units during differentiation of follicle cells in the *Drosophila* ovary. Intracellular pH is regulated by membrane ion transporters encoded by solute carrier (SLC) genes. We found that *dNhe2*, an SLC gene encoding a H+ efflux transporter that increases pHi, is required for prefollicle cell differentiation, and that overexpression of *dNhe2* causes follicle cells to prematurely adopt a differentiated fate. Collectively, our data support a model in which changes in pHi promote differentiation of the follicle stem cell lineage. Our preliminary experiments point toward a similar role for pHi in promoting differentiation in another type of stem cell lineage as well, suggesting that the process we are studying in follicle stem cells may be a more general feature of cellular differentiation. Future studies are aimed at understanding how an increase in pHi promotes differentiation by, for example, investigating whether proteins that promote cellular differentiation are regulated by the natural changes in pHi that we have observed. .

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Sex-lethal, Set1, and sexual identity in the adult female germline. Anne Smolko, Helen Salz. Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH.

Drosophila Sex-lethal (Sxl) encodes a female-specific RNA binding protein that regulates all aspects of female-specific development and behavior in somatic cells. *Sxl* also has a critical but less understood role in female germ cells. Germ cells without Sxl protein adopt a stem cell fate but fail to differentiate, continue to proliferate, and form a tumor. Recent studies in our lab demonstrate that tumor formation is accompanied by a global derepression of testis genes, indicating that germ cells without Sxl protein are masculinized. This conclusion is supported by our striking observation that the testis specific PHD finger protein 7 (Phf7), known to be required for male germline sexual identity, is inappropriately expressed in the absence of Sxl. Moreover, sex-inappropriate Phf7 expression is functionally connected to tumor formation, as oogenesis is rescued in germ cells lacking both Phf7 and Sxl protein, while forced expression of Phf7 in XX germ cells is sufficient to promote tumorigenesis. Phf7 is a known Histone 3 Lysine 4 (H3K4) methylation reader, suggesting a connection between maintenance of sexual identity and H3K4 methylation in the female germline. To investigate this possibility, we knocked down, *set1, trr,* and *trx,* the catalytic subunits of the three H3K4 methyltransferase complexes in the female germline. Surprisingly, of these three components, only *set1* is required for oogenesis. Our analysis reveals that loss of *set1* in the germline results in tumors with sex inappropriate expression of Phf7 and other testis genes. Results of genetic epistasis experiments further reveal that *set1* and *Sxl*work together to maintain sexual identity in the female germline. This suggests a model in which a failure in sexual memory is what drives tumorigenesis. .

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The role of the PIWI/piRNA pathway in the maintenance of genomic integrity. S. Mani, M. Zhong, N. Liu, H. Lin. Cell Biology, Yale University, New Haven, CT.

A hallmark of PIWI/piRNA pathway function is its requirement for fertility. The current model suggests that upregulation of transposons in *piwi* mutants causes activation of a DNA damage checkpoint leading to fertility defects. Alternatively, it is possible that *piwi* mutations directly affect genomic instability, independent of, or even leading to, increased transposon expression. In this study, we aim to understand what causes checkpoint activation by studying the developmental function of Argonaute3 (Ago3), a PIWI family protein.We first examined DNA damage in *ago3* mutants by studying the expression and localization of γH2Av, a marker of unrepaired double strand breaks (DSBs). An upregulation of γH2Av was observed in both germline and somatic cells despite only 'germline transposon' upregulation. Furthermore, an increase in γH2Av was seen in *ago3* heterozygotes, which do not exhibit increases in transposon levels. This suggests a dose sensitive effect of Ago3 on γH2Av that may not be correlated to transposon upregulation.To examine genome-wide DNA damage at high resolution, we devised a method to directly assess DSB formation. Preliminary data suggests that DNA damage on loss of Ago3 is increased in both gene and transposon regions. Furthermore, inactivation of the DNA damage checkpoint in *ago3* mutants partially rescued oogenesis, demonstrating an involvement of checkpoint activation in the observed defects. Checkpoint activation however produced a non-canonical response since no transcriptional change, cell cycle delay or

apoptosis was found. Interestingly, transposon levels are reduced in checkpoint-inactivated *ago3* mutants suggesting that transposon upregulation is downstream of checkpoint activation.Finally, spatial and temporal examination of DNA damage and transposon mRNA upregulation suggest that these two events might be uncoupled since DNA damage is seen early during oogenesis while transposon upregulation is more apparent at later stages.These observations represent the first detailed study of the relationship between DNA damage, transposon silencing, and the PIWI/piRNA pathway and suggest that transposon upregulation may not be the sole cause of compromised genome integrity in *piwi* mutants.

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Follicular adrenergic signaling plays an essential and conserved role in Drosophila ovulation. Lylah Deady[1], Jianjun Sun[1,2]. 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

The stress hormone norepinephrine (NE) plays a critical role in mammalian ovulation; however, the underlying cellular and molecular mechanisms remain controversial. It has been postulated that NE activates adrenergic receptors directly in the ovary to control ovulation or indirectly by regulating the secretion of luteinizing hormone. In contrast, the Drosophila equivalent of NE, octopamine (OA), is thought to control ovulation by regulating oviduct contraction and secretion. Therefore, it is not clear whether NE/OA regulates ovulation through a conserved mechanism. Recent work in our lab has shown that Drosophila ovulation resembles mammalian ovulation at both the cellular and molecular levels. During ovulation, posterior follicle cells (FCs) surrounding a mature oocyte are selectively degraded and the residual FCs remain in the ovary to form a corpus luteum-like body after the mature oocyte is ruptured into the oviduct. Like in mammals, this rupturing process also depends on Mmp2 activity. In the present study, we further investigated the role of OA signaling in ovulation. We found that OAMB (OA receptor) is expressed in mature FCs and that knocking down OAMB specifically in mature FCs hinders ovulation. We also showed that OA is sufficient to induce follicle rupture/ovulation independent of oviduct function but in a manner dependent on follicular OAMB and Mmp2. Furthermore, we show that OA's role in inducing ovulation can be replaced by NE. Our data strongly suggest that OA/NE activates follicular adrenergic signaling to control Mmp2 activity and ovulation and that this mechanism is likely conserved in mammals.

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A novel regulator of cell death, Bulsa, controls nuclear shrinkage during apoptosis. Yunsik Kang[1], Gina Castelvecchi[1,2], Doug Braun[1], Arash Bashirullah[1]. 1) University of Wisconsin-Madison, Madison, WI; 2) Washington University, St. Louis,MO.

An apoptotic cell has many signature features, including plasma membrane blebbing and nuclear shrinkage (a.k.a. pyknosis). However, the detailed mechanism of how pyknosis occurs during apoptosis is unknown. Here we identify a novel gene, *bulsa* ("immortal" in Korean) that is required for nuclear shrinkage during apoptosis. We identified 7 alleles of *bulsa* in a screen for novel regulators of apoptosis. *bulsa* mutant animals fail to remove obsolete tissues by programmed cell death during metamorphosis and die as late pupae. Loss of *bulsa* blocks nuclear shrinkage in cells otherwise fated to die, without blocking caspase activation. Conversely, ectopic expression of *bulsa* is sufficient to promote nuclear shrinkage without triggering caspase activation. We have conducted a modifier screen for suppressors of Bulsa-dependent pyknosis, and have outlined a pathway in which Bulsa drives nuclear shrinkage by regulating the nuclear actin-myosin cytoskeleton. Altogether, we demonstrate that Bulsa, a novel regulator of apoptosis, is necessary and sufficient for nuclear shrinkage during programmed cell death. These results highlight the essential role of pyknosis during cell death.

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Neural stem cell progeny regulate stem cell death in Notch and Hox dependent manner. Richa Arya, Tatevik Sarkissian, Kristin White. CBRC, MGH/HARVARD, CHARLESTOWN, MA.

Cell death is a prevalent, well-controlled and fundamental aspect of development. Despite an extensive understanding of the apoptotic machinery, there is much less known about the upstream regulators that activate the pathway in specific cells at the appropriate time in development. To examine the regulation of cell death in Drosophila, we have focused on a subset of neural stem cells that are eliminated by apoptosis during embryogenesis. In the absence of apoptosis, these stem cells continue to divide, resulting in a dramatically hyperplastic central nervous system and adult lethality. We are studying the spatial, temporal and cell identity cues that regulate the timely activation of cell death in these cells. We have identified a cis-regulatory region that controls the transcription of the cell death activators reaper, grim and sickle exclusively in neural stem cells. We performed an RNAi screen to identify the transcription factors that regulate neural stem cell death. These candidates fall in various functional categories from CNS development to chromatin remodelling. One strong candidate identified in this screen is Notch. Notch activity is required for neural stem cell death in the embryo. Notch regulates the expression of the abdominalA homeobox protein, which provides important spatial cues for death. Importantly, we show that pro-apoptotic Notch signaling is activated by the Delta ligand expressed on the neighboring progeny of the stem cell. Thus we identify a previously un-described role for progeny in regulating the proper developmental death of the parental stem cells.

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A Krebs cycle component and the mitochondria limit the rate of caspase activation during spermatid remodeling. Lior Aram, Carmel Braverman, Yosef Kaplan, Liat Ravid, **Eli Arama**. Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Partial cellular destruction during cell remodeling often involves active apoptotic caspases, but how cells avoid excessive caspase activity and unwanted cell death has been poorly understood. Here we use the caspase-mediated sperm remodeling process in *Drosophila* called "individualization" to investigate this striking phenomenon. We have identified an isoform of a Krebs cycle enzyme subunit, SCS-β_A, which binds to and activates the Cullin-3-based ubiquitin ligase (CRL3) complex required for caspase activation in spermatids. *In vitro* and *in vivo* evidence suggests that this interaction occurs on the mitochondrial surface, limiting the source of CRL3 complex activation and the consequent activation of caspases to the vicinity of this organelle. We further demonstrate an antagonistic interplay between this activating arm (SCS-β_A) and the inhibitory arm (Soti) of the CRL3 complex. Finally, we provide evidence that it is the structural role and not the Krebs cycle function of SCS-β_A which is important for spermatid individualization.

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Extracellular reactive oxygen species drive apoptosis-induced proliferation. Caitlin Fogarty, Jillian Lindblad, Neha Diwanji, Meghana Tare, Andreas Bergmann. Dept of Cancer Biology, University of Massachusetts Medical School, Worcester, MA.

Apoptosis-induced proliferation (AiP) is a compensatory mechanism to maintain tissue size and structure following unexpected cell loss during normal development. In apoptotic cells, caspase-initiated signaling cascades lead to the downstream production of mitogenic factors and the proliferation of neighboring healthy cells. In undifferentiated epithelial tissues, the Caspase-9 ortholog Dronc drives this form of compensatory proliferation via activation of Jun N-terminal kinase (JNK). Our lab has previously described genetic models of AiP that allow us to screen for modifiers and regulators of this pathway, but our understanding of the specific mechanisms leading to JNK activation remains incomplete. Here we show that Dronc-induced activation of JNK depends on the generation of extracellular reactive oxygen species (ROS) by dying cells. We have identified the NADPH oxidase family member, DUOX, as a contributor to these extracellular ROS, and important for the proliferative response following Dronc activation. Additionally, overexpression of catalases in the extracellular space inhibits JNK activation and downstream mitogen production. Interestingly, hemocyte-specific markers colocalize with ectopic JNK activity in the proliferating tissue. We propose that the ROS generated by dying cells attract and activate hemocytes, which in turn signal to activate the JNK cascade and drive AiP within the regenerating epithelium.

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The role of innate immune pathways in cell competition. Stefanie N Meyer[1], Marc Amyoel[2,3], Cora Bergantinos[2], Konrad Basler[1], Laura Johnston[2]. 1) University of Zurich, Zurich, Switzerland; 2) Columbia University, New York, NY USA; 3) New York University School of Medicine, NY, NY USA.

The function of a tissue, and thus the health of an organism can be compromised by the presence of mutant or weak cells. Cell competition is a mechanism that has evolved to prevent such cells from contributing to the tissue. Two widely studied models of cell competition are the Myc-induced super-competition, in which cells with increased levels of Myc outcompete (eliminate) neighboring wild-type cells and the competitive elimination of *Minute* cells. *Minute* are a class of mutants that predominantly carry a mutation in a ribosomal protein gene. *Minute* cells are viable, but when surrounded by wild-type cells they are eliminated. Relative cell vigor or fitness is thus believed to be a critical feature that is assessed in cell competition, however the mechanisms underlying the recognition and elimination the “less” fit cell are unknown. As this is reminiscent of what happens in the recognition and elimination of infected and damaged cells in the innate immune response, we studied if the innate immune response is required in cell competition.
In *Drosophila* two pathways govern the innate immune response: the IMD pathway, which leads to the activation of Rel, and the Toll receptor pathway, which activates Dorsal (dl) and Drosal related immunity factor (Dif). Rel, dl and Dif are NFκB homologs. We found that the Toll and the IMD pathway are important in cell competition and blocking them on various levels prevented the loser cells from being eliminated. We found the Toll-related receptors *Toll-3* and *Toll-9*, as well as the intracellular components *dl* and *Dif* to be required in the *Minute*-based cell competition. We also found that abrogating the function of *Dredd* or Rel by mutation or RNAi also rescued loser cells from death, so not only the Toll, but also the IMD pathway are important. We could also show, that the Toll and IMD pathway are important for the induction of apoptosis in cell competition by activating pro-apoptotic target genes. Thus we propose, that, the ancient innate immune response system is activated during cell competition and drives the elimination of loser cells.

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Fly-FUCCI - a versatile tool for studying cell proliferation in complex tissues. Norman Zielke, Jerome Korzelius, Monique van Straaten, Katharina Bender, Gregor Schuhknecht, Devanjali Dutta, Jinyi Xiang, Bruce Edgar. Deutsches Krebsforschungszentrum (DKFZ) - Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH) Allianz, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

The development of organs and tissues often involves strictly orchestrated lineages, in which only certain cell-types proliferate at a time. The decision to proliferate is highly dependent on signals from surrounding cells and hence one of the future challenges is to study cell proliferation within its microenvironment. The recently introduced FUCCI system (Fluorescent Ubiquitination-based Cell CycleIndicator) allows the monitoring of cell cycle phasing in living cells. To enable the specific labeling of small subpopulations of cells we have generated a fly-specific FUCCI system (Fly-FUCCI), whose expression can be spatially and temporally controlled. The Fly-FUCCI system is based on E2F1 and Cyclin B, which are sequentially degraded by the E3-ligases CRL4-Cdt2 and APC/C. Simultaneous expression of both Fly-FUCCI probes allows a distinction of all categories of interphase cells: G1 cells are marked by GFP/CFP; cells in S phase are labeled by RFP/YFP and cells in G2 express both markers. To support a broad range of experimental settings we have generated a toolkit of fly lines expressing the Fly-FUCCI probes under control of UASt, UASp and QUAS promoters. We demonstrate that the Fly-FUCCI system is capable of recapitulating the developmentally programmed cell cycle patterns in eye and wing discs. Furthermore, we have applied the Fly-FUCCI method to the stem cell lineage of the adult midgut, which revealed that the

terminally differentiated enterocytes re-enter the cycle during regeneration. Altogether, our work demonstrates that the Fly-FUCCI system is a valuable tool for visualizing cell cycle activity during development and tissue homeostasis. .

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Indispensable pre-mitotic endocycles promote aneuploidy in the *Drosophila* rectum. Kevin Schoenfelder[1], Ruth Montague[1], Sarah Paramore[1], Ashley Lennox[1], Anthony Mahowald[2], Donald Fox[1]. 1) Duke University, Durham, NC; 2) University of Chicago, Chicago, IL.

Polyploid cells, which contain extra sets of the genome, play vital roles in the development and physiology of diverse organs such as our heart and liver. Polyploidy is also observed with high frequency in many tumors, and division of polyploid tumor cells frequently creates aneuploidy (chromosomal imbalances), a hallmark of cancer. Despite this frequent occurrence of polyploidy and its association with aneuploidy, very little is known about the specific role that polyploidy plays in the diverse contexts under which it is found. Polyploid cells are frequently formed in an otherwise diploid organism via endocycles - alternate cell cycles during which the genome is duplicated but cell division never occurs. In my recently published work, I found that endocycles/polyploidy can enable tolerance of high levels of aneuploidy while also promoting proper organ development and function. Our lab previously discovered that the polyploid papillar cells of the *Drosophila* hindgut re-enter a traditional mitotic cell cycle after undergoing two rounds of the endocycle, and that these polyploid cell divisions are highly error-prone. Time-lapse studies of papillar polyploid mitosis revealed that the papillar cells underwent a high percentage of tripolar anaphase, which necessarily causes extreme aneuploidy. Despite this massive chromosome imbalance, I discovered that the tripolar daughter cells were both viable, and could promote normal organ development and function, suggesting that acquiring extra genome sets may enable a cell to tolerate the genomic alterations incurred by aneuploidy. Parallel studies are aimed at understanding the mechanistic relationship between undergoing an endocycle and papillar tissue development. The embryonic rectum is a one-cell-thick epithelium, which undergoes two endocycles during 2nd instar larval stage. During pupation, this epithelial monolayer simultaneously undergoes polyploid division and major morphogenetic events to form four cone-shaped adult papillae. Blocking the endocycle of the papillar cells results in major defects in organ morphology and function, indicating endocycles are an absolute requirement for proper papillar development. Future studies will further elucidate why the papillar cells utilize an error-prone polyploid mitosis for their development and how papillar cells deal with major aneuploidy.

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The *couch potato* gene mediates life history plasticity in *Drosophila melanogaster*. Katherine R. O'Brien[1,2], Paul S. Schmidt[2]. 1) Biology, University of Nebraska , Lincoln, NE; 2) Biology, University of Pennsylvania, Philadelphia, PA.

Despite the widespread occurrence of phenotypic plasticity, the genetic architecture and specific molecular variants that underlie plasticity are largely unknown. Genes that are pleiotropic and determine life history syndromes represent candidates for the mediation of adaptive plasticity. *Drosophila melanogaster* offers a rare opportunity to explore how specific genetic variants can affect phenotypic plasticity across multiple traits. A single nucleotide polymorphism at the gene *couch potato (cpo)* affects the expression of reproductive dormancy and a series of correlated, fitness-associated traits. This polymorphism also varies predictably in frequency in natural populations over multiple environmental gradients. In this study, the focal *cpo* variants were fixed in replicate natural genetic backgrounds to examine the effects of this polymorphism on plasticity in life history and behavior. Larval diet was manipulated to evaluate patterns of plasticity for the focal polymorphism and to assess whether patterns of plasticity were consistent across a series of integrative traits. The larval diet was iso-caloric media composed of food sources utilized by natural populations, either strawberries or apples, combined with high and low amounts of yeast. Our results demonstrate that a single polymorphism, previously associated with diapause plasticity, is also associated with differential expression of development time, mass, lipid content, food finding behavior and oviposition preference in response to variation in the larval environment. Interestingly, the low-diapause genotype was associated with a stronger response to rearing environment. This was unexpected because this allele is less plastic in response to photoperiod and thermal environments used to induce diapause. These data demonstrate the modular and complex nature of plasticity in which a single polymorphism governs plastic responses in distinct sets of phenotypes under different suites of environments.

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Investigating the molecular basis of species interactions: *Saccharomyces cerevisiae* mitochondria are required for optimal attractiveness to *Drosophila melanogaster*. Kelly Schiabor[1], Allison Quan[1], Michael Eisen[1,2]. 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, Berkeley, CA.

Drosophila and yeast interact in the wild, but the chemical basis of this interaction is unknown. While screening a collection of wild and laboratory yeast strains for their ability to attract adult *Drosophila melanogaster* (Raleigh 437), we noticed a large difference in fly preference for two nearly isogenic strains of *Saccharomyces cerevisiae*, BY4741 and BY4742. Using standard genetic analyses, we tracked the difference in preference to BY4742's lack of mitochondria in the initial experiment. We used gas chromatography coupled with mass spectrometry (GC-MS) to examine the volatile compounds produced by BY4741 and the mitochondria-deficient BY4742, and found that they differed in their production of known fly attractants. Specifically, we observed several ethyl and acetate esters (ethyl hexanoate, isoamyl acetate) at much higher levels in strains with mitochondria, even in fermentative conditions. By testing multiple synthetic substrates, we determined that the production of these compounds during sugar fermentation depends on the level and type of nitrogen in the yeast food. Yeast strains with mitochondria that ferment nitrogen-limited substrates produce the highest level of our identified ester attractants. This nutritional scenario – high sugar but limited nitrogen – matches the composition of fruit, the natural co-localization environment for flies and yeast, suggesting that these volatiles are ecologically relevant cues. Collectively these observations

demonstrate that core metabolic processes mediate the interaction between yeasts and insect vectors, and highlight the importance of mitochondrial functions in fly-yeast ecology. More generally, these results demonstrate the power of using two model systems, *D. melanogaster* and *S.cerevisiae*, to study the molecular basis of ecological interactions between species.

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Mitochondrial DNA x nuclear DNA interactions and environment modify fitness in the Drosophila Genetic Reference Panel (DGRP). Jim Mossman, Leann Biancani, Marissa Holmbeck, Lei Zhu, David Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

Mitochondrial and nuclear genetic interactions are vital for cell and organismal function. Crucially, when mtDNA and nDNA variants are in conflict, negative epistases can occur with sometimes severe deleterious effects. Here we describe a genetic system to investigate epistasis (G x G) and environmental modifiers (G x G x E) in the Drosophila Genetic Reference Panel (DGRP). We constructed 72 mito-nuclear genotypes from 12 DGRP backgrounds. Each DGRP line was introgressed onto three different *D. melanogaster* mtDNA haplotypes individually, or three different *D. simulans* mtDNA haplotypes individually. We hypothesized that inter-specific introgressions would confer deleterious phenotypes when compared to the intra-specific equivalents. We first tested all 72 genotypes on a standard food diet to investigate the role of mtDNA, nDNA and their interactions on development time and a proxy of fecundity (number of eggs laid). We found strong first order effects of nDNA and mtDNA genotype and strong second order interaction effects of mtDNA x nDNA combinations on both phenotypes. Moreover, there was considerable variation between DGRP lines in the amount of phenotypic variation among mtDNA haplotypes; some nuclear genotypes can tolerate introduced (inter-specific) mtDNA variation, others cannot. Interestingly, in some cases novel (inter-specific) pairings of mtDNA and nDNA generated highly fecund flies with low egg-to-adult development times, generating high fitness flies, contrary to expectation. We further tested the same genetic model in alternative food environments to test for G x G x E effects on development time. Using isocaloric food types, but with different protein and carbohydrate constituents, we found development time to be modified by diet; flies developed faster on high protein diets as expected. Moreover, there were individual cases of G x G x E, although these were not always in a predictable direction. Our lab is currently investigating the genetic basis of G x G x E using a combination of RNA-seq and forward genetic mapping techniques on flies reared in alternative environments.

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Contribution of sex, genotype, and environment to individual gene expression profiles. Kseniya Golovnina[1], Yanzhu Lin[2], Zhenxia Chen[1], Haiwang Yang[1], Hina Sultana[1], Brian Oliver[1], Susan Harbison[2]. 1) NIH/NIDDK, Bethesda, MD; 2) NIH/NHLBI, Bethesda, MD.

Personalized medicine is a major emerging theme in biomedical research. Sex, genotype, and environment are important factors to consider in developing treatments as even monozygotic human twins from the same family show phenotypic plasticity. To determine the scope of individual-to-individual variance at the steady-state transcription level and at the level of alternative splicing, we have produced a comprehensive single fly RNA-Seq data set on 768 individuals from 16 Drosophila Genetic Reference Panel (DGRP) inbred lines in triplicate "identical" environments. We observed subtle but highly significant expression differences in 95% of annotated genes due to sex. Males were also more sensitive to environmental differences, confirming and extending the idea that females and males deploy the genome in fundamentally different ways. SNPs, indels, and rearrangements in the 16 genotypes (the typical focus of GWAS studies) resulted in transcriptional variance in 67% of genes. Interestingly, 60% of genes showed differential expression among individuals grown on the same food and in the same incubator. Genes encoding proteins involved in redox reactions were the most variable, suggesting that stress responses vary greatly between individuals. This intriguing result shows high levels of expression plasticity among individual flies of identical genotype, suggesting their utility as a model for studying how plasticity arises. .

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The evolution of maternal mRNA deposition and zygotic genome activation across 14 *Drosophila* species. Joel Atallah, Susan E. Lott. Evolution & Ecology, University of California - Davis, Davis, CA.

Across the animal kingdom, the earliest embryonic stages are entirely regulated by RNA transcripts and proteins deposited by the mother, enabling development before the zygote is capable of transcribing its own RNA. At a critical stage, the embryo's genome is activated, zygotic transcription begins, and residual maternal transcripts are degraded, in a coordinated handoff of developmental control from the maternal to the zygotic genome. The maternal-zygotic transition, however, has only been studied in a few model systems, and very little is known about how it changes over evolutionary time. Here we present the first analysis of the evolution of the maternal-zygotic complement in closely related species. Using RNA-Seq, we probed the transcriptome of early embryos from 14 *Drosophila* species, at both a stage when all transcripts are maternal (stage 2) and a stage after zygotic genome activation (end of stage 5). We find numerous cases of evolutionary change, where orthologous genes are maternally deposited in one lineage and zygotically transcribed in another. Transitions in either direction usually occur through an evolutionary intermediate where the gene is both maternal and zygotic. We find that the mechanisms responsible for regulating these processes have themselves likely evolved. For example, specific DNA sequence motifs (such as the Zelda-bound TAGteam sites) are associated with the promoters of genes that are zygotically transcribed in *Drosophila melanogaster,* and are bound by proteins that promote transcription. However, a subset of these motifs are not associated with zygotic transcription in other species. Despite broad conservation of maternal and zygotic contributions to the embryo, the surprising number of evolutionary changes presents a unique opportunity to investigate both the evolution of developmental novelty and the mechanisms of gene regulation across developmental stages.

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Logic and mechanism of natural variation in the compound eye. Ariane Ramaekers[1], Simon Weinberger[1], Annelies Claeys[1], Erich Buchner[2], Reinhard Wolf[3], Bassem Hassan[1]. 1) VIB - KULeuven,Belgium; 2) University Hospital Würzburg, Germany; 3) University of Würzburg, Germany.

How variation in the development of the nervous system translates into morphological changes, and how, in turn, this affects its function is an open question. Variation in eye size is common between and within *Drosophila* species. In contrast, we observed that eye development is conserved: number of embryonic eye-antennal disc primordium cells, growth rate, and rate of progression of the morphogenetic furrow are strikingly shared despite millions of years of separate evolution. Thus, eye development may be strongly constrained – either due to developmental and genetic constraints or to selective pressure. Fly strains with different eye size presented a single developmental change: a modification of the proportion of the eye vs antennal field - within similarly sized eye-antennal discs. The same finding was true for intra and interspecific eye size variation, suggesting that changing eye to antennal field size ratio could be a favored route to eye size variation. This process requires mutual repression of eye- versus antenna- promoting transcription factors, such as Eyeless and Cut, respectively. We identified a single nucleotide polymorphism (SNP) correlating with variation in facet number between *D. melanogaster*lab strains. This SNP is located in a putative low affinity Cut binding site in a *cis*-regulatory module of *eyeless*, suggesting that a change in *eyeless* regulation could be at the origin of the variation in eye size, at least within *D. melanogaster*. Finally, we tested the functional consequences of facet number variation using a behavioral assay. By comparing visual acuity (spatial resolution) of "large" and "small" eye species, we observed that increased facet number is correlated with better acuity. This suggests a functional- and potentially adaptive – relevance of facet number variation.
In summary, this work bridges natural variation at the genetic, developmental, morphological and behavioral levels thereby shedding light onto the logic of variation in a complex and constrained system: the compound eye of insects.

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The molecular mechanism behind the evolution of a novel sex-specific trait. Gavin Rice[1], Olga Barmina[1], Michelle Arbeitman[2], Artyom Kopp[1]. 1) Evolution and Ecology, University of California at Davis, Davis, CA; 2) College of Medicine, Florida State University, Tallahassee, FL.

Although males and females share mostly the same genome, there are often traits with stark sexual dimorphism. Determining the molecular basis of how these traits are gained and modified in the course of evolution is vital for our understanding of the male and female form. It had long been thought that the sexual differentiation pathway is active in all cells and therefore was not under spatial or temporal regulation. However, the transcription factor *doublesex* (*dsx*) that regulates sex-specific gene expression in somatic cells is expressed in tightly restricted spatial patterns during *Drosophila* development. This leads to the hypothesis that for a new sexually dimorphic trait to evolve the expression pattern of *dsx* must be changed and that tissue specific, modular, *cis*-Regulatory Elements (CREs) in the *dsx* gene may be involved in the evolution of the sex-specific traits. The sex comb of *Drosophila* is an excellent model of a recently evolved, sex-specific morphological structure that has diversified rapidly and thus presents an ideal system to test this hypothesis. Furthermore, the proper development of sex comb is dependent upon localized expression of *dsx*, suggesting that sex comb evolution was caused, at least in part, by changes in *dsx* CREs. We have identified a 3kb region that is able to drive gene expression in the sex comb region. Furthermore, we have found that differences in the homologous CREs from different species are sufficient to change the expression of *dsx* in this region toward the pattern observed in the donor species. The expression of *dsx* induced by the *D. bipectinata* CRE transformed the *D. melanogaster* sex comb morphology, with a single row of sex comb teeth, to resemble the morphology of the *D. bipectinata* with two rows of sex combs. These results shed light on the role of CREs in the evolution of sexual dimorphism and novel traits.

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Talking Flies: Predator-induced changes in behavior can be socially communicated from exposed to naïve flies. Giovanni Bosco[1], Balint Kacsoh[1], Julianna Bozler[1], Mani Ramaswami[2]. 1) Genetics & Norris Cotton Cancer Ctr, Geisel Sch Med at Dartmouth, Hanover, NH; 2) Trinity College, Dublin, Ireland.

Behavioral adaptation to environmental threats and subsequent social transmission of adaptive behavior has evolutionary implications, particularly for reproductive behavior. In Drosophila, visual perception of parasitoid wasps leads to a sharp decline in fly oviposition, suggesting a possible neurobehavioral regulation of oogenesis in response to experiential cues. The molecular and neurogenetic basis for such experiential control of developing germline cells is not known. It is also not known whether such neuroendocrine control of oogenesis is innate, or if there is a learned response requiring memory consolidation that allows persistence of depressed oviposition through continued signaling from the brain to the ovary. We show that exposure to predatory wasps elicits both an acute and learned oviposition depression response. However, long-term persistence of oviposition depression after predator removal also requires neuronal cAMP signaling functions, neuronal translational control mediated by Orb2/CPEB shown to be essential for long-term memory, a functional mushroom body, and neurally driven apoptosis of oocytes through the caspases Dcp-1 and drice. Strikingly, wasp-exposed flies (teachers) can communicate egg-retention behavior and trigger ovarian apoptosis in naïve, unexposed flies (students) by communicating with wing movement or posture. Acquisition and behavioral execution of this socially learned behavior by naïve flies requires all of the factors needed for primary learning. Yet, the ability to teach does not require apoptosis in the ovary. This work provides new insight into genetic and physiological mechanisms that underlie an ecologically relevant form of learning, neuroendocrine control of germline development and mechanisms for its social transmission. We suggest this to be a new paradigm for studying social learning.

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Charaterization of a long-distance neurotransmitter recycling pathway essential for *Drosophila* visual transmission. Ratna Chaturvedi, Hong-Sheng Li. Department of Neurobiology , University of Massachusetts Medical School, Worcester, MA.

The continuation of many types of synaptic transmission in the central nervous system depends on glia-mediated neurotransmitter recycling. This recycling process was previsouly assumed to occur locally in the vicinity of synapse. However, our recently study in *Drosophila* visual system has revealed a long-distance mechanism of neurotransmitter recycling. In this novel mechanism carcinine, the recycling metabolite of the primary visual transmitter histamine, is transported from the neuropil region (lamina) via a multicellular glial network to retina, where it is taken up into the cell body of photoreceptor for histamine regeneration. Downregulation of a gap-junction protein Inx2 in larminar glia disrupted the photoreceptor transmission to laminar neurons, and impaired the fly vison in a behaviror assay. Here we identify a cation transporter in photoreceptor that may also participte in the histamine recycling. Knockdown of this transporter specifically in photoreceptors virtually blocked the photoreceptor transmission. In immunostaining, carcinine and its metabolites showed altered distribution in the retina and lamina of these knockdown flies, although the morphologies of retina and lamina remained intact. We hypothesize that this novel cation transporter may mediate carcinine uptake into photoreceptors during the long-distance recycling of histamine, which is essential to the visual transmission. Similar long-distance pathways of neurotransmitter recycling may functon in mammalian brains for sustained neuronal signal transmission.

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Maturation of central brain flight circuit in Drosophila requires Fz2/Ca^{2+} signaling. Tarjani Agrawal, Gaiti Hasan. NCBS-TIFR, Bangalore, India.

Flight behavior for flies is extremely essential for their survival and is controlled by various neuro-modulatory mechanisms, one of which is neuronal intracellular calcium signaling. Intracellular calcium signaling is triggered by activation of GPCRs on binding of its cognate ligand. I have performed an RNAi based modifier screen for GPCRs to find the ones that are essential for regulation of flight and function through intracellular calcium signaling. The identified GPCRs are found to have neuropepties as their cognate ligands, suggesting the neuro-modulatory role of neuropeptides to drive flight. An interesting finding from the GPCR RNAi screen suggests a role for the non-canonical Fz2/Ca^{2+} signaling pathway in flight circuit maturation. In the past there have been speculations for the Fz2 mediated calcium signaling, however no concrete study showing any physiological relevance have been done so far. Finding the role of Fz2/Calcium in regulation of flight gave us opportunity to probe this further. We have been able to show that Fz2/calcium signaling is required during development in dopaminergic neurons of the central nervous system to regulate flight. This Fz2 signaling is found to be purely non-canonical and requires active participation of G-protein molecule Go for its function during flight. Also, Fz2 mediated calcium signaling is required for maintaining the optimum levels of tyrosine hydroxylase and neuronal activity in specific set of dopaminergic neurons called PAM neurons. Optimum levels of tyrosine hydroxylase in adult neurons and neuronal activity during development are required for adequate flight behavior.

Identification of central brain flight circuit neurons and their dopaminergic properties enables us to further study the downstream neurons that play a role in air-puff driven flight. This will help us to dissect out the neuronal circuit with its neurotransmitter and electrophysiological properties to control flight in *Drosophila*.

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Systematic characterization of sensorimotor transformations in the Drosophila larva. Luis A. Hernandez Nunez, Mason Klein, Lindsey Claus, Aravinthan Samuel. Department of Physics and Center for Brain Science, Harvard University, Cambridge, MA.

Understanding how sensory neuron signals are transformed into motor responses is a central question in neuroscience; however, the difficulty of controlling the activity of specific neurons while monitoring and quantifying the behavior of large numbers of animals has hampered the efforts made to answer it. Here we use the *Drosophila melanogaster* larva to present a systematic approach that allowed us to circumvent these difficulties. We engineered a high throughput experimental setup capable of inducing random activity in targeted neurons in large numbers of freely moving animals. We used machine vision algorithms and reverse correlation analysis to identify which neuron activity patterns trigger a specific behavioral response. We applied this strategy to characterize how individual sets of peripheral sensory neurons modulate navigation. This powerful approach can be readily extended to study other neurons of the *Drosophila* larva and of other transparent animals.

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***Drosophila* exercise-training requires octopaminergic neuron activity. Alyson Sujkowski**, Robert Wessells. Wayne State School of Medicine, Detroit, MI.

Endurance exercise is a promising therapeutic intervention with substantial protective effects on multiple indices of healthspan, including muscle and cardiac function. Male *Drosophila* respond to a ramped daily program of exercise by inducing conserved physiological responses similar to those seen in mice and humans. Female flies induce negative geotaxis but fail to sustain strong behavioral response during the training period. As a result, females do not experience the adaptive training response seen in males. Here, we demonstrate that poor female exercise response is mediated by differences in neuronal activity, not by differences in muscle. We also show that the sex-specific exercise-training response is reversible even in adults, after development has been completed. Using tissue-autonomous expression of sex determination constructs, we have identified octopaminergic neurons as sufficient to govern adult

exercise behavior. Importantly, manipulating octopaminergic neurons is also sufficient to induce conserved cellular and physiological changes seen following endurance training. Reversal of sex-specific response can also be accomplished by providing octopamine or octopamine antagonist in food. Octopamine, the invertebrate ortholog of norepinephrine, is an important neuropeptide involved in diverse behaviors as well as learning, memory and reward. This model provides an important opportunity to further examine the specific pathways mediating reward-based behavioral and physiological changes.

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Sensorimotor transformation of cooling in the *Drosophila* larva. M. Klein[1], B. Afonso[1,2], M. Berck[1], L. Hernandez-Nunez[1], L. Ni[3], M. Zlatic[2], P.A. Garrity[3], A.D.T. Samuel[1]. 1) Dept. of Physics and Center for Brain Science, Harvard University, Cambridge, MA; 2) HHMI Janelia Farm Research Campus, Ashburn, VA; 3) Dept. of Biology, Brandeis University, Waltham, MA.

Complex animal behaviors are built from dynamical relationships between sensory inputs, neuronal activity, and motor outputs in patterns with strategic value. Connecting these patterns illuminates how nervous systems compute behavior. Here, we study *Drosophila* larva navigation away from cold temperatures (positive thermotaxis). By tracking the movements of animals responding to fixed spatial temperature gradients or random temperature fluctuations, we calculate the sensitivity and dynamics of the conversion of thermosensory inputs into motor responses. We uncover three thermosensory neurons in each dorsal organ ganglion (DOG) that are required for positive thermotaxis. Random optogenetic stimulation of the DOG thermosensory neurons evokes behavioral patterns that mimic the response to temperature variations. *In vivo* calcium and voltage imaging reveals that the DOG thermosensory neurons exhibit activity patterns with sensitivity and dynamics matched to the behavioral response. EM reconstruction of sensory and brain circuitry also illuminates second order circuitry and points toward a more complete understanding of the processing of cooling response information in the larva. .

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An RNAi/CRISPR screen for novel regulators of synaptic development and function. C. Dustin Rubinstein[1], Caley A. Hogan[2], Nathan J. Carpenter[1], Emily M. Fong[1], Grace Heglund-Lohman[1], Anna E. Zeidman[1,3], Gene H. Thiede[1], Kate M. O'Connor-Giles[1,4]. 1) Laboratory of Cell and Molecular Biology, U. of Wisconsin-Madison, Madison, WI; 2) Genetics Training Program, U. of Wisconsin-Madison, Madison, WI; 3) IBS-SRP, U. of Wisconsin-Madison, Madison, WI; 4) Department of Genetics, U. of Wisconsin-Madison, Madison, WI.

Although the Drosophila synapse is a well-studied model system, many genes affecting synaptic development and physiology have likely thus far escaped elucidation. We sought to identify novel synaptic regulators using a combination of bioinformatics, high-throughput screening, and genome engineering. We generated a candidate gene list for screening by identifying genes with a developmental expression pattern exhibited by many canonical synaptic genes. We refined our list to genes that have not been previously characterized, genes that are expressed neuronally, genes that have identifiable vertebrate homologs, and genes that have readily available RNAi reagents, which yielded a list of 74 genes. We reasoned that if a candidate gene is a synaptic regulator, disrupting its expression should affect synapse morphology, decrease synaptic transmission, or alter the behavioral output of synaptic assemblies. To identify such genes, we knocked down expression via RNAi and assayed synaptic growth at the larval neuromuscular junction, synaptic transmission using electroretinograms, and adult locomotor behavior. Our analysis yielded 12 genes exhibiting altered synaptic growth, 10 genes with synaptic transmission defects, and 11 genes with locomotor deficits. These genes included cell-surface signaling molecules, GPCRs, and protein kinases. We have focused on the four genes that exhibited mutant phenotypes in more than one assay. We have generated loss-of-function alleles using CRISPR-Cas9 genome engineering for confirmation of RNAi hits in the same assays and for future detailed mechanistic studies. The results of our screen demonstrate how RNAi screening, which is rapid but prone to off-target effects, can be enhanced by bioinformatics and strengthened by highly efficient CRISPR-Cas9 genome engineering to identify high-confidence regulators of nervous system development and function. .

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***Krüppel* expression levels are maintained through compensatory evolution of shadow enhancers. Zeba Wunderlich**[1], Meghan D. J. Bragdon[1], Ben J. Vincent[1], Jonathan White[2], Angela H. DePace[1]. 1) Systems Biol, Harvard Med Sch, Boston, MA; 2) Swarthmore College, Swarthmore, PA.

Compensatory evolution describes the compensation of a single deleterious mutation by another, thereby yielding a neutrally-fit double mutant. It has been shown that compensatory evolution is responsible for conserving the function of individual orthologous enhancers across large phylogenetic distances. In *Drosophila,* many developmental genes are controlled by shadow enhancers, pairs of enhancers that drive overlapping expression patterns. In these cases, we hypothesize that compensatory evolution can maintain the total expression pattern of a gene of interest while allowing individual shadow enhancers to diverge. To test this hypothesis, we analyzed expression driven by orthologous pairs shadow enhancers that control expression of *Krüppel*, a key transcription factor in anterior-posterior axis patterning in Drosophila embryos. We measured the expression patterns of reporter constructs consisting of the two embryonic *Kr* enhancers singly and in combination from two *Drosophila* species, *D. melanogaster*and *D. pseudoobscura*. We find that the expression driven by the pair of enhancers is conserved between these two species, but expression patterns driven by individual enhancers are not. This result suggests that compensatory evolution acts to maintain the overall expression pattern driven by both shadow enhancers. Using sequence analysis and experimental perturbation, we demonstrate that each shadow enhancer is controlled by different transcription factors. These results demonstrate that compensatory evolution can occur at the level of a gene locus.

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Evolution of the Novel Gene Zeus. Robert Arthur[1,3], Benjamin Krinsky[1,2], Kevin White[1,2,4], Manyuan Long[1]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 3) Committee on Evolutionary Biology, University of Chicago, Chicago, IL; 4) Department of Human Genetics, University of Chicago, Chicago, IL.

Recent work has demonstrated that young, or newly evolved, genes are not only ubiquitous across the tree of life, but also that these new genes can rapidly acquire novel functions that influence a diverse array of biological processes. Previous work identified a young gene in *Drosophila*, *Zeus*, which diverged rapidly from its parent *Caf40* and took on roles in the male reproductive system. Binding profiling of the Zeus protein from *Drosophila melanogaster* revealed this transition was accompanied by differential binding to loci throughout the genome. Herein, we use a novel comparative framework to explore the evolution of binding profiles of Zeus and Caf40 proteins from multiple species. By comparing Zeus ChIP-seq data from *D. melanogaster* and *D. simulans*, as well as Caf40 from *D. yakuba*, a dynamic pattern emerges in which Zeus rapidly acquired and lost targets between species. Interestingly, while both copies of Zeus acquired targets at male-biased and testis-specific genes, *D. melanogaster* and *D. simulans* proteins have specialized on different chromosomes. We have also discovered a motif associated with Zeus, which appears to have undergone strong positive selection since the origination of the Zeus protein. Taken together, our results suggest a complex pattern by which Zeus acquired sex-specific functions through a unique combination of neo- and sub-functionalization, and affected a genome-wide change in the selective regime of its binding motif.

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Mapping genetic background effects on transvection. Teresa Rzezniczak, Thomas J. S. Merritt. Department of Chemistry & Biochemistry, Laurentian University, Sudbury, Ontario, Canada.

Genetic background often affects the phenotypic manifestation of a mutation, although some phenotypes are more drastically affected than others. The somatic pairing of homologous chromosomes, and the effects of this pairing on gene expression (transvection), are widespread in *D. melanogaster*, but relatively little is known about the effects of genetic backgrounds on this pairing. Using a pair of knockout alleles, we quantified the amount of transvection at *Malic enzyme* (*Men*) in each of the Drosophila Genetic Reference Panel (DGRP) lines to address this question. As has been observed in other complex phenotypes, we found a wide range of variation in the amount of transvection across the DGRP backgrounds. Interestingly, the amount of transvection for each background was often significantly different between *Men* alleles, suggesting there is an allele x background effect. Further, we also found that several of the DGRP lines showed no transvection at *Men*, suggesting the disruption of pairing possibly by uncharacterized chromosomal inversions in these DGRP lines. We performed a genome wide association study to identify modifiers of transvection at *Men* and compared these to modifiers of*Men* enzyme activity and kinetics. Genetic background trends in this dataset were compared and contrasted with genetic background trends in other species using a database we have compiled. .

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Intragenomic conflict and satellite DNA evolution in *Drosophila*. Amanda M Larracuente, Daniel E Khost, Anthony Geneva. Department of Biology, University of Rochester, Rochester, NY.

The rapid evolution of satellite DNA—the large blocks of tandem repeats found at centromeres and telomeres— can contribute to genomic instability and genetic incompatibilities between species. Despite its abundance in eukaryotic genomes and its contribution to genome evolution, we know little about the dynamics of satellite DNA evolution. Progress towards developing statistical methods to ask about forces driving the evolution of satellite DNAs at the level of genomes has been stymied by the difficulty in assembling repeats and employing accurate mutation and recombination models. We developed a phylogenomic approach that circumvents these issues, using Next Generation Sequencing data to estimate the diversity and abundance of satellite repeats in genomes. We use our methods to study the evolution of the *Responder* (*Rsp*) satellite DNA family in the *melanogaster* subgroup. *Rsp* is well-known for being the target of *Segregation Distorter* (*SD*)— an autosomal meiotic drive system in *D. melanogaster*. We show that *Rsp* family evolution is highly dynamic over a short evolutionary time scale (<240,000 years) and we detect genomic signatures that may shed light on the causes of their expansion and contraction across *Drosophila* lineages. We also show that *Rsp* has a history of accelerated evolution in *D. melanogaster*, where it is a target of meiotic drive.

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P-element invasion of *Drosophila simulans*. Andrea Betancourt, Tom Hill, Robert Kofler, Viola Nolte, Christian Schlötterer. Institute for Populationsgenetik, Vetmeduni Vienna, Vienna, Austria.

We survey a world-wide sample of *Drosophila simulans* for evidence of hybrid dysgenesis, a syndrome of defects characteristic of crosses with transposable element activity unrepressed by the female parent. We found evidence of dysgenesis between *D. simulans* collected from different locations and at different times, suggesting that these strains differ in at least one important active transposable element. Using genome sequencing data and PCR, we found only one of 58 active transposable elements showed differences between strains consistent with the cause of dysgenesis. This was the P-element, not previously known from *D. simulans*, but the classic example of a dysgenesis causing element in *D. melanogaster*. The P-element appears to have been recently acquired in *D. simulans* from *D. melanogaster*, and to have spread rapidly world-wide, with strains from the same geographic region showing P-element to have increased from low to high frequency within a decade. .

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African and European admixture in southeast US and Caribbean Islands populations of *Drosophila melanogaster* affect postmating reproductive phenotypes. Joyce Kao[1,2], Asif Zubair[1], Matthew Salomon[1], Seana Lymer[1,2], Sea Hwang[1], Albert Sung[1], Daniel Campo[1], Sergey Nuzhdin[1]. 1) University of Southern California, Los Angeles, CA; 2) New York University, New York, NY.

The current demographic model of *Drosophila melanogaster* states that flies originated in Africa with an expansion into the European continent 10,000 years ago. Colonization of the Americas is hypothesized to have occurred in two waves: African flies arriving in the Caribbean Islands with the transatlantic slave trade and cosmopolitan flies arriving with European settlers into North America. These two waves created a hybrid zone in the southeast United States (SE US) and Caribbean Islands of cosmopolitan-adapted flies from Europe and African-like flies from West Africa, which do show phenotypic and behavioral differences. Recent pooled sequencing endeavors of North American fly populations report African alleles not found in the European cosmopolitan flies as well as African and European admixture in east coast populations. These findings support the current hypothesis of the colonization of the Americas, but more evidence is needed to complete the scenario involving recent African admixture into American flies and its consequences. We have sequenced 23 individual *D. melanogaster* genomes from various locations in the SE US and the Caribbean Islands to explore this more recent admixture. We show African and European admixture extends from the southeast US into the Caribbean Island with a southward clinal trend of increasing African ancestry and distinct admixture inheritance patterns across the genome in these populations. In addition to population genomic analysis, we also investigated post-mating reproductive barriers and show decreased fecundity in flies originating from the border of the SE US and Caribbean Islands, which is the interface where the most African and European admixture is occurring. We looked at female longevity after mating to males of different origins and also found a possible increase in resistance to sperm toxicity in these highly admixed flies. Our results not only expose the source of previously reported novel African alleles in east coast US populations, but also illustrate some of the reproductive consequences of genomic admixture.

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Natural Selection Shapes the Mosaic Ancestry of the *Drosophila* Genetic Reference Panel and the *D. melanogaster* Reference Genome. John Pool. Laboratory of Genetics, University of Wisconsin - Madison, Madison, WI.

North American populations of *Drosophila melanogaster* are thought to derive from two sources, with a primary genetic contribution from Europe and a secondary contribution from the sub-Saharan ancestral range of the species. Despite the importance of North American strains for genetic research, patterns of European and African admixture along their genomes (and the evolutionary forces shaping them) have remained entirely unknown. In this study, I reveal geographic ancestry along genomes of the *Drosophila* Genetic Reference Panel (DGRP) and the *D. melanogaster* reference genome. Overall, the proportion of African ancestry was estimated to be 20% for the DGRP and 9% for the reference genome. However, ancestry levels varied strikingly across these genomes. Very little African introgression was observed on the X chromosome, congruent with patterns observed in many other admixing populations and hybridizing taxa. DGRP ancestry levels also varied dramatically within chromosome arms, with considerably more African admixture in regions of lower recombination rate. Among the functional categories of genes most enriched for European ancestry was "circadian rhythm". A strong genome-wide signal of "ancestry disequilibrium" was also observed, in that many between-chromosome pairs of genomic windows showed a deficiency of Africa-Europe allele combinations. These results support the hypothesis that admixture between partially genetically isolated *Drosophila* populations has led to epistatic natural selection against incompatible genetic variants, and that this process is ongoing. The ancestry blocks inferred here will bolster the design and interpretation of many population genetic and association mapping studies.

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Three-step mechanism for the spatial and cell-cycle dynamics of pericentric chromatin. Eric Joyce, Tharanga Senaratne, Ting Wu. Department of Genetics, Harvard Medical School, Boston, MA.

In *Drosophila*, as in many organisms, centromeres are clustered into nuclear bodies called chromocenters that, along with their proximal heterochromatin, form a repressive nuclear compartment. Notably, the role of this repressive compartment in genome stability has been greatly substantiated in recent years, with evidence for its impact in many processes, including heterochromatin and transposon silencing, DNA repair, and chromosome segregation. However, the spatial organization of centromeres can differ significantly among cell types, and our understanding of the molecular factors that control their positioning remains largely unknown. To this end, we have utilized our high-throughput technology for fluorescent *in situ* hybridization (Hi-FISH) in combination with RNAi to directly interrogate centromere positioning in a systematic fashion. We will present the results of this screen, which, collectively, provide evidence for three distinct and important steps in centromere positioning – establishment, maintenance, and re-organization. In particular, we identified kinetochore and centrosome components as factors important for centromere clustering, providing a mechanistic link between mitotic chromosome segregation and interphase nuclear architecture. In addition, we have discovered a programmed disruption of centromere clustering and spatial re-organization of these chromatin domains that is driven by the condensin II complex. We propose a simple model for how the activity of these factors can be modulated to alter levels of centromere clustering and heterochromatic silencing events across different cell-cycle stages, tissues, and developmental time points. The relationships between centromere positioning and global nuclear organization, cell cycle progression, and chromatin compaction will also be discussed. This work is supported by grants from the National Institutes of Health to E.F.J. (F32CA157188) and T.W. (RO1GM61936 and 5DP1GM106412).

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Mrg15-dependent binding of Cap-H2 to chromatin is required for chromosome organization and regulation of gene expression. Heather Wallace[1], Huy Nguyen[1], Julianna Bozler[1], Gregory Rogers[2], Giovanni Bosco[1]. 1) Genetics, Geisel School of Medicine at Dartmouth, Hanover , NH; 2) Department of Cellular and Molecular Medicine University of Arizona, Tucson, Arizona.

The spatial organization of the genome within the eukaryotic nucleus is a dynamic process that plays a critical role in gene expression, DNA replication, and chromosome segregation. Condensins are conserved multi-subunit protein complexes that contribute to chromosome organization by regulating chromosome compaction and homolog pairing. Previous work in our lab has shown that the CAP-H2 subunit of condensin II physically and genetically interacts with the *Drosophila* homolog of human MORF4-related gene on chromosome 15 (MRG15). Like CAP-H2, MRG15 is required for interphase chromosome compaction and homolog pairing. However, the mechanism by which MRG15 and CAP-H2 cooperate to maintain interphase chromatin organization remains unclear. Here we show that CAP-H2 localizes to interband regions on polytene chromosomes in a manner partially dependent on MRG15. We have identified a binding motif within CAP-H2 that is essential for its interaction with MRG15, and have found that mutation of this motif partially suppresses CAP-H2-mediated compaction and homolog unpairing. Consistent with previously published ChIP-seq data, we observed that CAP-H2 co-localizes with MRG15 at regions of active transcription and is enriched at telomeres of polytene chromosomes. We show that mutation of *Cap-H2* leads to altered localization of telomeric capping proteins on polytene chromosomes, and we observed telomere fusions in mitotic chromosome spreads from S2 cells treated with *Cap-H2* RNAi. RNA-seq in cells depleted of CAP-H2 showed altered levels of expression for approximately 4000 genes, a majority of which were down-regulated more than 2-fold. We propose a model in which MRG15 acts as a loading factor to facilitate Cap-H2 binding to chromatin and mediate changes in chromatin organization and gene transcription.

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Trimethylation of Histone H3 at lysine 27 by Polycomb Repressive Complex 2 and its role in epigenetic memory. Rory T. Coleman, Gary Struhl. Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY.

During the development of most animals, the body is subdivided into distinct segmental primordia specified by the early establishment of heritable ON/OFF codes of HOX genes belonging to the Antennapedia and Bithorax complexes. How cells in any given segment "remember" the OFF state of the appropriate HOX genes thereafter is a central problem in animal development. The products of the Polycomb Group (PcG) gene family, notably those that form the components of Polycomb Repressive Complex 2 (PRC2), which mediates trimethylation of Histone H3 at lysine 27 (H3K27me3), are known to play a critical role in this process. However, the molecular mechanism by which the repressed state is transmitted through cell division remains elusive. Using a transgenic reporter of the classical HOX gene Ultrabithorax, we show that when the PcG proteins can no longer be anchored to the transgene by cis-acting Polycomb Response Elements (PREs), the OFF state can be maintained indefinitely in the absence of replication, but is progressively lost in dividing cells, in a manner that depends on the number of rounds of cell division. Analysis of the reporter chromatin shows that release from the silenced state is correlated with dilution in the levels of H3K27 trimethylation. Our results suggest that the epigenetic memory of the OFF state of HOX gene expression may be explained by this capacity for H3K27 trimethylated nucleosomes to be inherited through cell division.

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Tip60 HAT Action in Environmental Enrichment induced Cognitive Restoration. Songjun Xu. Biology, Drexel University, Philadelphia, PA.

Environmental enrichment (EE) conditions have profound beneficial effects for reinstating cognitive ability in neuropathological conditions such as Alzheimer's disease (AD). While EE benefits involve epigenetic gene control mechanisms that comprise histone acetylation, the select HATs involved remain largely unknown. Here, we test the hypothesis that Tip60 HAT action is required for a beneficial neuroadaptatvie response to EE. We use the mushroom body (MB) as our cognitive model as this neural circuit in the adult fly brain is where Tip60 is robustly produced, is central for learning & memory (L&M), and exhibits beneficial morphological changes in response to EE. We initially tested whether EE promotes beneficial changes on MB morphology under AD conditions and whether this response is dependent upon Tip60 HAT action. To test this, we simultaneously tag MB cells with GFP while increasing Tip60 HAT levels in the MB under AD conditions. Newly eclosed adult fly progeny were exposed to EE or isolation (ISO) conditions. Remarkably, we observed a beneficial axonal outgrowth neuroadaptative response to EE in the AD MB that was dampened in comparison to control w^{1118} flies and restored by excess Tip60. Our results indicate that appropriate levels of Tip60 HAT activity are required for EE mediated neuroadaptative morphological benefits, and that excess Tip60 alleviates impairment of an EE response in AD flies. To test mechanisms underlying Tip60 involvement in EE rescue, we asked whether Tip60 HAT action is required to induce a *neuroadaptative transcriptional response* to EE. We induced Tip60 HAT loss in the MB while tagging MB cells with GFP. Newly eclosed adult fly progeny were exposed to EE or ISO conditions. We then FACs purified GFP tagged MB Kenyon neurons from conditioned fly brains for detection of an EE induced MB transcriptional response. Bioinformatics analysis of microarray data from EE vs ISO is indicative of a neuroadaptative transcriptional response to EE. Together, our studies are the *first* to demonstrate that the fly MB undergoes a beneficial neuroadaptive transcriptional response to EE and suggests that Tip60 HAT action is involved in this process. .

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Dietary restriction reduces transposable element expression in aging *Drosophila* heads. Jason G. Wood, Brian C. Jones, Nan Jiang, Stephen L. Helfand. Dept. of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI.

Transposable Elements (TEs) are mobile genetic elements that constitute a large percentage of the DNA content of eukaryotic genomes. Because of their ability to disrupt gene expression and cause genomic instability, cells have evolved methods of silencing expression and mobilization of TEs, including the establishment of repressive heterochromatin around TEs. The aging process is characterized by a breakdown in homeostatic mechanisms, including loss of genomic integrity. We have previously shown that aging is characterized by loss of repressive heterochromatin structure in pericentromeric regions as well as chromosome 4. Specifically, characteristic H3K9me3 and HP1 marks present in these regions decline with age. We examined expression with age of genes that are natively located within these heterochromatic regions, and observed a number of these genes whose expression increased with age in the head of *Drosophila melanogaster*. Because TEs are especially prevalent within heterochromatic regions, and due to the deleterious effects of TE expression and mobilization, we also examined whether aberrant TE expression was a hallmark of aging. We found that expression of numerous TEs increased with age in the head, suggesting a loss of heterochromatin structure can lead to TE mobilization. Dietary restriction is an environmental intervention that reliably extends lifespan in numerous organisms, including flies. In addition, dietary restriction normally delays a number of age-related dysfunctions and pathologies. We observed that a dietary restriction regimen repressed the increased expression of both native heterochromatin genes as well as TEs that we observed during normal aging. Together, these data suggest that proper maintenance of repressive heterochromatin structure may be important for prevention of the deleterious effects of aging.

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A novel chromatin factor Enhancer of Polycomb acts in somatic cells to maintain germ cell identity and activity in Drosophila adult testis. Lijuan Feng, Zhen Shi, Xin Chen. Biology, Johns Hopkins University, Baltimore, MD.

As one of the best characterized adult stem cell systems, two types of adult stem cells, germline stem cells (GSCs) and cyst stem cells (CySCs), reside in the testicular niche of Drosophila. The CySC lineage had been thought to play a supportive role for germ cell proliferation and differentiation. But how CySC maintenance and differentiation are regulated as well as how this regulation is coordinated with the germline lineage remain poorly understood. Here we show that a Polycomb group component Enhancer of Polycomb [E(Pc)] is required for CySC differentiation; and in turn promote germ cells differentiation. E(Pc) is also required to maintain germ cell identity. Loss of this critical chromatin regulator in somatic cells leads to germ cell overproliferation and expression of somatic cell marker. To understand the molecular mechanism of E(Pc), we performed Chromatin Immunoprecipitation combined with massive parallel sequencing (ChIP-seq) to specifically identify E(Pc)-targets in somatic cells of Drosophila testis. E(Pc) is found to enrich at many important genes known to be functional in somatic gonadal cells. Analysis of E(Pc) targets not only confirm some known regulatory mechanism coordinating CySC and GSC lineages, such as EGFR signaling; but also shed light on some new mechanisms. For example, we identified *yan* as a direct target of E(Pc). Genetic data demonstrate that loss of E(Pc) led to ectopic Yan expression and reducing Yan suppressed *E(Pc)* phenotype, suggesting that Yan is negatively regulated by E(Pc). E(Pc) has been shown to be a component of the Tip60 histone acetyltransferase complex. Interestingly, *Tip60* is also a direct target of E(Pc) by ChIP-seq assay. In this scenario, E(Pc) acts as an activator for promoting *Tip60* expression and *Tip60* knockdown resulted in similar phenotype compared to *E(Pc)* phenotype. Our genomic and genetic data show that E(Pc) can act as both a transcriptional repressor and activator, and both functions are important to regulate CySC differentiation to coordinate with GSC differentiation and also antagonize somatic identity in germ cells.

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Impacts of centromere misregulation on genome stability and cancer progression in a Drosophila model of glioblastoma. Nicole Beier[1,2], Renee Read[3], Gary Karpen[1,2]. 1) Department of Molecular and Cell Biology, University of California Berkeley; 2) Genome Sciences Division, Lawrence Berkeley National Laboratory; 3) Emory University.

Centromeres are regions of eukaryotic chromosomes essential for proper kinetochore formation and chromosome segregation, and are maintained epigenetically through cellular divisions and the germline by the presence of the centromere-specific histone H3 variant CENP-A (CID in Drosophila). Recent studies have identified proteins required for proper CENP-A localization and function. In flies, the chaperone/assembly factor CAL1 and the inner centromere protein CENP-C are co-required for CID assembly and function. Centromeric proteins are commonly misregulated in many types of human cancers Overexpression of centromere proteins may be a mechanism for the creation or maintenance of chromosomal instability and aneuploidy in cancer cells, or may contribute to genomic or transcriptional changes that contribute to cancer progression. Previous studies in flies have focused on CID misregulation in cultured cells. Here, we use a Drosophila cancer model system to investigate the effect of misregulation of CID and its assembly/stability partner CAL1 on cell division and genome instability in animal tissues, and additionally demonstrate the impact of centromere misregulation on cancer initiation and progression.

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Physiological connectivity and inter-clock coupling in the *Drosophila* circadian clock neuron network. Zepeng Yao, Jenna Clem, Amy Bennett, Orie Shafer. Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI.

Circadian rhythms have evolved in most organisms to predict and cope with daily environmental changes. In animals including *Drosophila*, a network of circadian clock neurons in the brain controls most physiological and behavioral rhythms, including daily sleep/activity rhythms. How such a network of clock neurons is connected and coordinated to generate coherent outputs remains

an important and unanswered question in the field. We have recently developed a new approach for functional neural circuit analysis in the fly brain, which combines controlled "chemogenetic" excitation of upstream neurons with simultaneous monitoring of putative target neuron responses using genetically encoded fluorescent sensors. Using this approach, we have demonstrated a functional connection between the ventral lateral clock neurons (LN_vs, regarded as the "Morning" cells) and the dorsal lateral clock neurons (LN_ds, regarded as the "Evening" cells) via the neuropeptide pigment-dispersing factor (PDF). We are now using this method to investigate the connectivity among other classes of clock neurons.
To study molecular clock coupling between clock neurons, we have genetically sped-up or slowed-down the molecular clock in subsets of clock neurons, and measured the changes of molecular oscillations in other clock neuron classes using time-course immunocytochemistry with single-cell resolution. Our results indicate that the clock network is organized into multiple discrete oscillatory units, each unified by the neuropeptide(s) it expresses and its unique mode of coupling to other units. For instance, we find that the PDF-expressing LN_vs are capable of delaying a unique subset of LN_ds via PDF signaling, but not capable of advancing them. In contrast, a different class of clock neurons, the posterior dorsal neurons 1 (DN_{1p}s), can be both advanced and delayed by the LN_vs. We are currently investigating the mechanisms of such differential inter-clock coupling, and the potential roles it might play in seasonal adaptation.

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Dynamic central neuron activities that underlie courtship pursuit in *Drosophila* male. S. Kohatsu, D. Yamamoto. Graduate School of Life Sciences, Tohoku University, Sendai, Japan.

In *Drosophila melanogaster*, cellular substrates of the male courtship behavior have been extensively studied, yet our knowledge on their physiological properties are limited. To identify central neuron activities that are correlated with particular behavioral process of courtship, we optically measured calcium activities from interneurons expressing a sex determination gene *doublesex* (*dsx*)in a male placed on a treadmill. By providing concecutive stimuli of female contact and computer-generated horizontal visual motion stimulus, we successfully induced courtship pursuit and wing extension/vibration, two of the hallmarks of normal male courtship, in a male during *in vivo* funcional imaging. In the lateral protocerebrum, we detected multiple, transient calcium rises, which coincided with courtship pursuit and wing extension/vibration. Mosaic analysis revealed that pC1 neurons, a class of male-specific interneurons known to mediate courtship initiation, are responsible for the calcium activities observed during courtship pursuit. The calcium rises that occurred in the pC1 neurons were selectively associated with locomotion turns of the fly toward the side where the somata of the recorded neurons were located in the brain. It is suggested that pC1 neurons dynamically control the direction as well as activity level of courtship pursuit relying on pheromonal and visual cues.

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Previous socio-sexual experience modulates the mating investmentof male *Drosophila melanogaster*. Woo Jae Kim[1], Yuh Nung Jan[2], Lily Yeh Jan[2]. 1) Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada; 2) Howard Hughes Medical Institute, Departments of Physiology, Biochemistry, and Biophysics, University of California San Francisco, San Francisco, California 94158, USA.

Environmental conditions, such as the socio-sexual context, can change rapidly. In these circumstances, the ability of animal of a genotype to express a different phenotype according to the environment can improve survival and reproductive success. A primary function of males for many species involves mating with females for reproduction. For males, current mating investment can often be subject to trade-offs with future mating opportunities to maximize fitness in a rapidly changing socio-sexual environment. *Drosophila* males respond to the presence of rivals by prolonging mating duration to increase the chance of passing on genes. In previous studies, we examined the genetic network and neural circuits that regulate rival-induced longer mating duration (LMD). LMD can be induced solely via visual stimuli. LMD depends on the circadian clock genes *timeless* and *period*, but not *Clock* or *cycle*. LMD involves the memory circuit of the ellipsoid body (EB). Further, we identified a small subset of clock neurons in the male brain that regulate LMD via neuropeptide signaling. (*Kim et al., Nat. neuro, 2012; Neuron, 2013*). More recently, we investigated whether sexual experience could affect males' investment in mating. We found that sexually experienced males shortened mating duration compared to naïve males (shorter mating duration, SMD). Both sexual experiences and contact-based chemoreception is necessary to induce SMD. We identified sexually dimorphic *Gr5a*-positive "sugar cells" as important for sensory input. In contrast to LMD, SMD depends on the circadian clock genes *Clock* and *cycle*, but not *timeless* or *period*. SMD requires the function of short neuropeptide F (sNPF), but not PDF or NPF. Our study delineates part of the molecular and cellular basis of plastic behaviors elicited by different socio-sexual environments. LMD together with SMD can be a model to study how the socio-sexual environment affects on male behavioral plasticity via neural circuitry.

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Physiological and behavioural correlates of natural variation in an insect olfactory receptor. Katherine H Shaw[1,2], Alisha Anderson[2], Marien de Bruyne[1], Coral G Warr[1]. 1) Biological Sciences, Monash University, Clayton, VIC, Australia; 2) Ecosystems Sciences, CSIRO, Black Mountain, ACT, Australia.

The responses of flies to many volatile cues are determined by a family of olfactory receptor (Or) proteins. In *Drosophila,* response profiles of olfactory receptor neurons are generally conserved between species, but one, the ab3A neuron, shows high levels of divergence. *Or22a*, the gene that determines ab3A response, shows variation in copy number and sequence across *Drosophila* species. There are two copies of this gene in laboratory strains of *D. melanogaster*, *Or22a* and *Or22b*, with the latter thought to be non-

functional. A single hybrid receptor, *Or22del*, has also been found at this locus in natural populations, consisting of *Or22b* with its N terminus replaced with that of *Or22a*. The *Or22del* allele shows clinal variation in frequency; it is present at high frequency in northern parts of Australia, and absent in the south. We investigated whether this allele affects ab3A response and olfactory behaviour. We bred a number of isochromosomal lines of flies from northern and southern populations, recorded their ab3A response to a range of odorants, and found three different response profiles. One of the phenotypes correlates with the presence of an *Or22a* allele with similar sequence and response to the one seen in our laboratory stock Canton-S. A second phenotype correlates with *Or22del,* and by expressing this gene in empty ab3A neurons we proved that *Or22del* mediates this phenotypic change. Using a choice assay, we also show that the behavioural response to selected odorants depends on whether *Or22del* or *Or22a* is present. Thus a change in olfactory-driven behaviour may underlie the apparent selection for *Or22del* we see from its cline in frequency. We also made constructs to study the effect of the amino acid changes observed in Or22del on the function of Or22b, and found a single amino acid change that reconstituted a functional Or22b. Interestingly, this allele shows a response similar to the third phenotype we had found. Examination of ab3A responses of closely-related species suggests that this third phenotype is the ancestral one, providing insight into the evolution of this receptor.

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New Pheromones and Olfactory Receptor Pathways Mediating Behavior in Larvae. Jonathan T Clark, Anandasankar Ray. University of California, Riverside, Riverside, CA.

Chemical communication plays a major role in the social behavior of insects. Pheromones are species-specific chemicals released by organisms to trigger social responses and are some of the most powerful elicitors of behavior known. Despite the relative simplicity of the larval olfactory system, little investigation into pheromone-driven behavior in larvae has been conducted. However, pheromonal communication in larvae is of interest because social behaviors such as aggregation are implicated in larval fitness. Using a chemical ecology screening approach, we identified a few behaviorally active compounds in larvae. Larvae demonstrated attraction to two compounds, and aversion to one compound. The attraction responses are lost in larvae mutant for the olfactory receptor co-receptor (*Orco*), suggesting that the *odorant receptor* (*Or*) family is involved. Using mutant lines, we were able to identify a single *Or* for at least one of these compounds that causes robust attraction. This attraction is lost in the *Or* mutant across a range of concentrations. Electrophysiological analysis of the pheromone-receptor pair revealed a significant increase in spike frequency that was lost in the *Or* mutants. We propose that this receptor plays a role in larval aggregation behavior, leading to improved foraging in offspring. Our results report new receptors and circuits that respond to fly odors and provide a sophisticated template to analyze how pheromones, whether alone or with food odors, generate behavior.

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Innexin7 containing gap junctions in *Drosophila* Antennal Lobe Projection Neurons contribute to synchronous neuronal activity and olfactory responses. Jorge M Campusano[1], Nicolás Fuenzalida-Uribe[1], Bryon Silva[1], Diane K O'Dowd[2]. 1) Pontificia Universidad Católica de Chile, Santiago, Chile; 2) Dept. Developmental and Cell Biology, University of California Irvine.

In the mammalian olfactory bulb (OB), gap junctions synchronize mitral and tufted cell activity during processing of olfactory information. It has been suggested that gap junctions are also present in the insect Antennal Lobe (AL), a structure which is functionally homologous to the mammalian OB. Recent studies in *Drosophila* indicate that the invertebrate gap junction protein innexin 8 (inx8) contributes to electrical synapses between AL projections neurons (PNs). While other innexins including innexin 7 (inx7) are also expressed in the insect AL, little is known about their functional role. Here, by using the calcium imaging technique, we show that AL PNs in culture display spontaneous calcium transients. When two PNs are in physical contact, the calcium transients of these neurons are highly synchronized. RNAi knockdown of inx7 ($RNAi^{inx7}$) in PNs reduces the synchronization of spontaneous calcium transients observed in pairs constituted by these neurons. Furthermore, our data show that expression of $RNAi^{inx7}$ in PNs perturbs behavioral responses to two different odorants, Octanol and Methylcyclohexanol. Interestingly, no difference in behavioral responses to a different odorant, benzaldehyde, is detected. These results suggest that inx7-encoded gap junctions help coordinate PN activity and olfactory information processing in the adult *Drosophila* AL.

Grant Fondecyt 1141233 (JMC)

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Photoreceptor neurotransduction requires a conserved Ncc69 activating kinase cascade specifically in glia. Drew Stenesen[1], Jeffrey Schellinger[2], Aylin Rodan[2], Helmut Krämer[1]. 1) Neuroscience, UT Southwestern Medical Center, Dallas, TX; 2) Internal Medicine, UT Southwestern Medical Center, Dallas, TX.

Both human and *Drosophila* nervous systems function under precise ionic and osmotic control. Loss of this homeostatic regulation has direct clinical implications related to seizure, coma, and brain injury. Despite this importance the molecular mechanisms that maintain ion environments within the brain remain unclear. Here, we examine the role and regulation of the Na^+-K^+-$2Cl^-$ symporter, Ncc69, using the*Drosophila* visual system as a model of neuronal function. A large deletion within the Ncc69 genomic locus blocks visual transduction as indicated by the absence of electroretinogram "ON"- and "OFF" transient components. Surprisingly, Ncc69 localizes to laminal glia within the visual system, and Ncc69 expression in glia, but not neurons, is both necessary and sufficient for proper visual transduction. Ncc69 transport activity is known to be activated by direct binding and phosphorylation from the serine/threonine kinase Fray, which itself is activated by phosphorylation from *Drosophila* WNK (With No lysine=K) kinase. Glial-specific

loss of either WNK or Fray phenocopies the phototransduction defects of Ncc69 mutants. Genetic rescue experiments with mammalian orthologs of WNK suggest a conserved physiological mechanism potentially involving the Cl^- binding site within the WNK kinase domain. Taken together, we have established a three-protein cascade functioning within glia to regulate intracellular chloride concentrations that are critical for proper neuronal function.

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Male Costs of Reproduction are Self-Imposed and Mediated by Perception of the Opposite Sex. Zachary Harvanek[1], Emily Feuka[1], Sean Kelly[1,2], Scott Pletcher[1]. 1) Mol/Int Physiology, Univ. of Michigan, Ann Arbor, MI; 2) Univ. of Rochester, Rochester, NY.

Evolutionary theories that incorporate costs of reproduction generally posit that reproductive behaviors and physiology are energetically costly or physically risky, resulting in trade-offs between offspring production and life history traits such as health and longevity. Our laboratory has demonstrated that exposure of males to female pheromones (without mating) decreases lifespan and alters physiology of males through the pheromone receptor *ppk23*. Therefore, we hypothesized that the costs of reproduction may be self-imposed through perception of the opposite sex. Thus, we established an experimental paradigm where mating was uncoupled from perception, allowing us to determine whether mating itself is a costly act. We exposed experimental males to cohorts of "donor" flies that included wild-type males or females, or transgenic females that expressed male pheromones (these "masculinized females" were otherwise normal) to independently manipulate mating and pheromone exposure. We observed that elimination of female pheromones also eliminated all measured costs of reproduction: males exposed to masculinized females had higher fat stores, greater starvation resistance, and significantly longer lifespan than males exposed to wild-type females, and they were indistinguishable from males exposed to other wild-type males. To determine whether preventing perception of female pheromones could extend lifespan of males in mixed sex populations, we examined *ppk23* mutants in our paradigm. Indeed, mutation of *ppk23* extended lifespan only in males exposed to wild-type females. In all cases, we confirmed that experimental males mated similarly with masculinized and wild-type females, suggesting that perception of the opposite sex orchestrates self-imposed physiologic changes that compromise overall health and well-being. Investigation of the mechanisms underlying these changes may provide insight as to why males initiate these seemingly self-destructive programs, and ongoing experiments have implicated germline signaling and the neuropeptides Corazonin and Akh. This research opens the door for studying the costs of reproduction not as a set of physically imposed trade-offs, but instead as a regulated physiologic process.

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Tissue specific orchestration of nutrient dependent responses in *D. Melanogaster*. Guiping Du, Patrick Wai-Lun Li, Artem Zykovich, Kazutaka Akagi, Sean D. Mooney, Simon Melov, Pankaj Kapahi. Buck Institute for Research on Aging, Novato, CA.

Dietary restriction (DR) is a robust method for extending health-span across species. Previous DR-regulated gene expression studies in flies have been done at the whole organism level, which fails to take into account the importance of tissue-specificity. We have developed a method to effectively isolate cell type-specific ribosome bound mRNAs using tagged ribosomes in *Drosophila melanogaster*. Using multiple tissue-specific GAL4 drivers, the ribotag approach achieved reliable results that are consistent with those obtained by dissection. We investigated translational profiles in different tissues (including neuron, germ line, muscle, heart, gut, fat body and Malpighian tubule) upon DR in *Drosophila*. A large number of genes regulating signal transduction including secreted factors and ligands are elevated, whereas genes involved in cell proliferation are decreased upon DR. We observed tissue specific signatures of responses to DR. In the neurons, DR enhanced neurotransmission and the production of neurotransmitters and neuropeptides, some of which are required for DR-related lifespan extension. Muscle and heart function was optimized in response to DR as shown with elevation of genes regulating sarcomere organization and myofibril assembly. On the other hand, germ line function is compromised by DR as shown with reduction of protein complexes including cell cycle checkpoint and DNA replication. Interestingly, nuclear encoded mitochondrial genes were increased in the gut and fat body while these genes were downregulated in other tissues like the brain. Paradoxically the gut and fat body showed an increase in ribosomal genes involved in mRNA translation while other tissues had an expected decrease in these factors. Genes regulating translational initiation, elongation, and tRNA synthetase were also increased in the gut. Consistent with this finding, activation of TOR signaling in the gut extends lifespan on rich nutrient diet. Our results demonstrate a distinct tissue specific response which can sometimes even be paradoxical in different tissues in response to DR in flies. Various tissues orchestrate these responses to optimize the function of the whole organism to extend lifespan.

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A novel role for Dpp as an endocrine signal potentially linking growth status to the regulation of ecdysteroidogenesis and developmental progression. Linda Setiawan, Iswar K. Hariharan. Department of Molecular and Cell Biology, University California Berkeley, Berkeley, CA.

For many organisms, post-embryonic developmental transitions correlate with the attainment of a specific size. For *Drosophila* larvae the shift from a growth phase to the onset of metamorphosis is dependent upon achieving a "critical weight". Metamorphosis is then activated by increased production of the steroid hormone ecdysone, by the prothoracic gland (PG). The mechanism by which the PG assesses the weight of the organism is not known. While manipulating the size of the PG itself can affect the timing of pupariation, it is also likely that humoral factors communicate the growth status of the remainder of the organism to the PG.
We have found that the morphogen Dpp, which has a well-characterized role in patterning imaginal discs, has an unexpected function as a long-range signal. Dpp can diffuse from imaginal discs to the PG and regulate the timing of pupariation. In the PG, Dpp signaling inhibits expression of several "Halloween genes", which encode enzymes that function in ecdysone biosynthesis. During normal

progression of larvae through the third instar, the level of Dpp signaling in the PG decreases thus alleviating repression of the Halloween genes and promoting ecdysone production.
By what mechanism could the level of Dpp signaling in the PG be regulated in an age-dependent manner? One possibility is that the quantity of secreted Dpp, the "source", decreases throughout the third instar, for example due to altered proteolytic cleavage or changes in properties of the extracellular matrix. Alternatively, growing tissues could function as a "sink" and reduce the abundance of circulating Dpp. We propose a scenario, in which the attainment of "critical weight" is characterized by a reduction in circulating Dpp levels to an extent that the PG can produce sufficient ecdysone to initiate pupariation.

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Metabolic processes implicated in developmental robustness under thermal stress. Steven G. Kuntz[1], Anthony T. Iavarone[1], Kelly M. Schiabor[2], Peter A. Combs[3], Michael B. Eisen[1,4]. 1) QB3, UC Berkeley, CA; 2) Molecular and Cell Biology, UC Berkeley, CA; 3) Biophysics Grad Group, UC Berkeley, CA; 4) HHMI, Berkeley, CA.

Drosophila develop normally over a wide range of temperatures, coordinating numerous molecular and cellular processes that each scale independently with temperature. We previously demonstrated that, while the total duration of embryogenesis varies 3-fold across species and temperature, the relative timing of all major morphological events during embryogenesis is always the same. Our current research focuses on identifying the molecular mechanisms driving this remarkable developmental isomorphy.
Using precise temperature control and careful morphological staging, we analyzed the expression and metabolic profiles of developing embryos across a wide range of temperatures (17.5-30°C) and investigated maternal effects. Using mass spectrometry we find as temperature increases, the concentrations of certain phosphatidylinositols, phosphatidylglycerols, and phosphatidylethanolamines decrease in the embryo, while 1,1-dimethylguanidine and butyraldehyde both increase. We observe exceptional similarity in expression profiles across temperatures, but find that a small number of transcriptionally-associated factors, including *Thor* and *SNCF*, are up-regulated in the early embryo as temperatures increase while other DNA-binding proteins and kinases, including *HmgD* and *awd*, are down-regulated. Maternal heat-conditioning (25°C) may be important for this process, as the response is reduced in embryos from cold-conditioned (18°C) mothers, along with expression of some ribosomal and transcriptional proteins, such as *RpS27A* and *Ef2b*. By investigating the expression profiles of gravid mothers, we demonstrate that heat-conditioning leads to the up-regulation of many vitellin and chorion membrane proteins, which may assist the embryo during heat stress. Our results suggest that metabolic processes play a key role in thermal responses. We will also present data from ongoing experiments using pharmacological manipulation during temperature-controlled time-lapse imaging to investigate the specific role of transcriptional, translational and other metabolic processes in the developmental response to thermal changes.

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A new mechanism of sexual differentiation controls sexually dimorphic physiology in the adult intestine. Bruno Hudry, Irene Miguel-Aliaga. MRC Clinical Sciences Centre, Imperial College London, London, United Kingdom.

Male and female animals often differ in their metabolism and physiology. Sex determination factors are known to play a key role in the formation of reproductive organs and secondary sexual characteristics, which could indirectly lead to physiological differences between the sexes. However, it is currently unclear whether sex determination factors remain active in adult somatic organs. We have used the adult *Drosophila* midgut to explore the nature and physiological significance of adult sexual dimorphisms. RNAseq analysis of male and female adult midguts uncovers a considerable number of sexually dimorphic transcripts coding for proteins with roles in proliferation, growth and metabolism. Combined with intestinal cell type-specific manipulations of the sex determination pathway, transcriptional profiling indicates that many sexual dimorphisms are cell-type specific, and can be abrogated by adult-specific, cell-autonomous interference with sex determination factors. We then focus on one cell subpopulation, the intestinal stem cells (ISCs), to characterize the molecular mechanisms involved and to establish their functional significance. Clonal and adult specific-manipulations of ISC sexual identity reveal a non-canonical sex determination pathway comprising Sex Lethal and Transformer (Tra) but, unexpectedly, not Doublesex or Fruitless. Genetic and transcriptional analyses of sexually dimorphic splicing events indicate that Tra mediates the female and stem cell-specific splicing of a secreted growth factor. We find that, at the cellular level, the sex determination pathway does not affect the viability or division mode of ISCs, but does shorten the duration of the cell cycle in female ISCs, thus speeding up divisions. Furthermore, this adult-specific sexual dimorphism in mitotic potential has multiple functional consequences at the organ and organism levels, both during normal homeostasis and in situations of abnormal proliferation. Together, our findings show that adult somatic cells express their sex cell-autonomously through a novel and physiologically significant mechanism of sexual differentiation. They also indicate that sexual dimorphisms are not confined to Doublesex and Fruitless-expressing cells.

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Activinβ/TGF-β signaling in skeletal muscle controls insulin signaling and metabolism to influence final body size. Lindsay Moss-Taylor[1], Michael O'Connor[2]. 1) Molecular, Cellular, Developmental Biology, and Genetics Program, University of Minnesota, Minneapolis, MN; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Inter-organ communication is essential for regulating development and homeostasis. Mutations in Drosophila *Activin-Beta* (*Actβ*) cause accelerated pupariation and reduced final body and organ size. To determine how *Actβ* affects size and timing, we first looked at which cells express *Actβ* and found expression in the Insulin Producing Cells (IPCs), neuroendocrine cells and motor neurons. Overexpression of *Actβ* in either neuroendocrine cells or motor neurons increases body size. Muscle-specific knockdown of the TGF-β signaling transducer/transcription factor *dSmad2* reduces body size, indicating muscle is a target tissue of the Actβ signal. Additionally,

levels of phospho-dSmad2 are reduced in skeletal muscle samples of *Actβ* mutants and increased in animals overexpressing *Actβ* from motor neurons. Levels of phospho-S6K are correlate with phospho-dSmad2 levels, suggesting TGF-Beta signaling in muscle regulates insulin signaling. Because insulin signaling controls metabolism, we used GC/MS analysis to identify and quantify levels of metabolites in whole-larval samples of *Actβ* mutants. We found intermediates of the energy-producing steps of glycolysis and lactic acid are reduced, indicating reduced flux through glycolysis. Overall, this indicates neuronally-derived Actβ signals to the skeletal muscle to regulate levels of insulin signaling and subsequent glycolysis. We have identified over 300 downstream targets of dSmad2 using skeletal-muscle RNAseq and will test potential target genes using tissue-specific knock-downs to determine how TGF-Beta signaling influences insulin signaling and how the muscle coordinates body growth and final organ size.

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Let-7 Overexpression Extends Longevity and Alters Fat Metabolism in Female *Drosophila Melanogaster*. Christi Gendron, Scott Pletcher. The Geriatric Center and the Molecular and Integrative Physiology Department, University of Michigan, Ann Arbor, MI.

The goal of this study was to identify specific microRNAs (miRNAs) that significantly impact adult-specific longevity and fat metabolism. An initial screen using *Drosophila melanogaster* identified the *let-7-complex* (containing the miRNAs *let-7*, *miR-100*, and *miR-125*) as a candidate. Therefore, our objective became three-fold: (1) to confirm that the *let-7-complex* influences both organismal longevity and fat metabolism, (2) to identify which member(s) of the *let-7-complex* were responsible for these changes, and (3) to identify the specific tissues where overexpression of individual *let-7-complex*members were sufficient to drive longevity extension and altered fat metabolism. We confirmed that whole body, adult-specific *let-7-complex* overexpression resulted in significant increases in both lifespan and fat stores compared to control flies. This effect was seen only in female *D. melanogaster*. Whole body overexpression of individual *let-7 complex* members did not recapitulate the lifespan extension seen with overexpression of the entire *let-7-complex*, suggesting that there is an interaction between *let-7-complex* members that drives lifespan extension. However, overexpression of *let-7* did cause increased fat stores. Tissue specific overexpression of individual *let-7-complex* members revealed that *let-7* itself, when overexpressed in nervous tissue, causes a substantial increase female longevity. In conclusion, we identified the *let-7-complex* as having the ability to extend fly longevity and alter fly metabolism, suggesting an important role for these miRNAs in the aging process. Surprisingly, *miR-125*overexpression, which has been shown to increase longevity in *C. elegans*, did not increase fly lifespan. Rather, overexpression of *let-7* alone in the fly nervous system caused the largest increases in longevity observed and implicates a critical role for this miRNA in promoting lifespan by down-regulating the expression of molecules that accelerate the aging process. Further work is ongoing to identify the mechanisms behind these observations.

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Regulation of protein consumption by an ovarian peptide transporter. Sonali Deshpande, William Ja. Metabolism and Aging, The Scripps Research Institute, Jupiter, FL.

Animals can regulate their consumption based on the nutritional value of available food. Although both protein and carbohydrate elicit compensatory feeding behavior, the effect is not solely dependent on calories since protein induces greater satiety than equicaloric carbohydrates. However, mechanisms underlying protein-sensing remain elusive. In this study, we use a radioactive tracer to assay food intake and determine that short peptides, rather than whole protein or amino acids, are the most efficient at eliciting compensatory feeding in *Drosophila* adults. Using RNAi targeting homologs of known peptide transporters belonging to the PTR2 or the ABC transporter family, we found that ubiquitous down-regulation of several genes diminishes the compensatory feeding response to changes in dietary protein concentration. One of these predicted peptide transporters, CG7627, is enriched in ovaries—a major site for protein utilization in females. Ovary-specific knock-down of CG7627 shifts preference for protein and carbohydrate intake. Our results indicate involvement of an ovarian gene in food intake, increasing our knowledge regarding molecular mechanisms regulating feeding. Studies of peptide transporters, which are highly conserved in mammals, may provide new insights on treating feeding-related metabolic disorders.

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***Muscle migration is directed by a combination of intrinsic polarity and short-range signaling*. Elly Ordan**[1], Marko Brankatschk[2], Barry Dickson[3], Frank Schnorrer[4], Talila Volk[1]. 1) molecular genetics, Weizmann Institute of Sc1-Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israelience, Rehovot, Israel; 2) 2-Institute of Molecular Pathology (IMP), A-1030 Vienna, Austria. Present address: Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 3) Institute of Molecular Pathology (IMP), A-1030 Vienna, Austria; 4) 4-Max Planck Institute of Biochemistry, 82152 Martinsried, Germany.

The current theory of how muscle elongation is directed is based on concepts borrowed from neuronal migration. Study of this process has led us to propose that, unlike neurons, muscles react primarily to short-range signals that correct their path, which is based on an intrinsic directionality. The repulsive protein Slit secreted from tendons has been previously proposed as a long-range attractant for muscle migration. However, our findings demonstrate that through tight control of its distribution, Slit repulsion is used for both directing and arresting muscle migration towards tendon cells. We show that Slit cleavage restricts its distribution to tendon cells, allowing it to function as a short-range repellent directing muscle migration and patterning and promoting their halt upon reaching the target site. Mechanistically, our findings demonstrate that Slit processing produces a rapidly degraded C-terminal fragment and an active, stable N-terminal polypeptide that is tethered to the tendon cell membrane, which further protects it from degradation. Consistently, the requirement for Slit processing can be bypassed by providing an uncleavable, membrane-bound form of Slit that is stable and is retained on expressing tendon cells. Moreover, muscle elongation appears to be extremely sensitive to Slit levels, as

replacing the entire full-length Slit with the stable Slit-N-polypeptide results in excessive repulsion, which leads to defective muscle pattern. These findings reveal a novel cleavage-dependent regulatory mechanism controlling Slit spatial distribution, which may operate in other Slit-dependent processes.

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Understanding EGFR activation in patterning the proximal-distal axis of the *Drosophila* leg. S. Tozier[1], R. Voutev[2], R. S. Mann[2]. 1) Biological Sciences, Columbia University, New York, NY; 2) Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

The evolution of appendages represents a major advance in the ability of animals to interact with their environment. The molecular mechanisms governing growth and patterning of appendages also represents a significant addition to the developmental repertoire of animals. In fruit flies, the proximal-to-distal axis of the leg is established during larval development when cooperative signalling by two morphogens, Dpp and Wingless (Wg), activates concentric circular domains of gene expression in the leg imaginal disc. These circles are further subdivided into domains that prefigure the adult leg segments by the expression of several transcription factors activated by a central gradient of EGFR signalling. The specific regulatory mechanisms responsible for activating this gradient in the proper spatio-temporal pattern have not been described. We have identified leg enhancers of genes encoding two key inputs for EGFR activation: the neuregulin-like ligand Vein and the serine protease Rhomboid, required for processing of the other, TGF-α-like EGFR ligands. Mosaic analysis supports a role for the downstream effectors of Wg and Dpp signalling as well as the transcription factors Distalless (Dll) and Sp1 as candidate regulators of EGFR inputs. These genetic experiments combined with site-directed mutagenesis of putative binding sites for these regulators in the enhancers of *vein* and *rhomboid* reveal that these EGFR inputs are regulated by independent, but overlapping transcriptional programs. Both enhancers require direct input from the homeobox TF Dll, but differ in their specific requirements for Wg and Dpp input, via TCF and Mad binding. *vein*, but not *rhomboid*, requires input from the zinc-finger TF Sp1, a leg selector gene. Differential regulation of the two avenues into EGFR activation likely confers robustness on this key developmental process. Moreover, because EGFR is used repeatedly during development to mediate many varied processes, the ability to compare and contrast multiple regulatory paradigms provides insight into how organisms can produce specific outcomes from a general signal.

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Nemo kinase: a multi-step regulator of planar cell polarity. Giovanna M Collu[1], Konstantin Gaengel[1], Ivana Mirkovic[1], Wang A Yanfeng[1], Mei-Ling Chin[1], Andreas Jenny[2], Marek Mlodzik[1]. 1) Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY; 2) Department of Developmental and Molecular Biology Department of Genetics Albert Einstein College of Medicine.

Frizzled-Strabismus/Van Gogh Planar Cell Polarity (PCP) signaling regulates patterning of the Drosophila eye through directing cell fate specification and collective cell rearrangement. We have previously shown that Nemo kinase is required to couple the core PCP factors Strabismus and Prickle-Spiny legs to the E-cadherin-β-catenin complex to drive ommatidial rotation. Here we investigate an earlier requirement for Nemo kinase activity in photoreceptor fate specification within the developing eye disc.
The main phenotype in *nemo* mutant eye tissue is misrotation, however we discovered that *nemo* genetically interacts with PCP core factors to enhance the chirality defects associated with R3/R3 photoreceptor misspecification. In particular loss of *nemo* function strongly enhances the pk^{sple} chirality phenotype, resulting in appearance of many symmetrical clusters. Furthermore, *nemo* genetically interacts with pk^{sple} in the leg, enhancing the spiny leg phenotype with an increase in ectopic joint tissue. Through mosaic analysis in the eye, we show that Nemo is required in R4 and not R3, suggesting that Nemo promotes Pk-sple function. We use biochemical assays to demonstrate Nemo phosphorylates Pk and to map the phosphorylation sites to two clusters within the C-terminal domain. Our data suggest that Nemo phosphorylates Pk-sple and promotes Pk-sple function. Thus PCP-coupled Nemo activity is required to direct individual photoreceptor fate specification in addition to regulating rotation of the whole ommatidial cluster. .

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***Notch*-Dependent Tissue Folding Determines Boundary between Developmental Fields. Hui-Yu Ku**[1,2], Y. Henry Sun[1,2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

Fate-determined cells are tightly controlled in cell proliferation, rearrangement, and morphogenesis in order to achieved organized and precise structures during development. *Drosophila* larval imaginal disc has been a valuable tool to explore the underlying mechanisms responsible for boundary formation. *Drosophila* adult head is derived from the larval eye-antennal disc (EAD), each organ primordium in the EAD can be identified by the expression of regional identity genes. However, mechanisms to maintain sharp expressing domains of these genes and restrict lineage distributions remain unclear. Mechanical tensions have been shown to prevent cell intermingling across boundaries. We find that sharp gene expression boundaries coincided with tissue folding in the EAD. By analyzing twin-spot mitotic clones (Twin-spot MARCM) induced at various stages, the clonal distributions indicate that cell-lineages respect tissue folding. Mutant clones defective in folding do not maintain sharp expression boundaries of regional identity genes in the EAD. Mixtures of differentiated cells are observed in mutant adult heads. Chromophore-assisted laser inactivation (CALI) combined with clonal tracking reveal that cells no longer stay within original territory once folding is disrupted in the EAD culture. Loss or gain of N activation recapitulates folding defect and mixture of regional identity genes, or causes ectopic tissue folding, respectively. N activation is required in apical constriction during the folding process. We purposed that N triggers tissue folding between different primordia, and tissue folding serves as a lineage-restricted boundary to prevent cell intermingling, ensure correct partition of developing EAD. .

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Systematic analysis of protein-RNA interactions in *Drosophila*. John Laver[1], Xiao Li[1], Hong Na[1], Juhyun Jeon[1], Fateh Singh[1], Timothy Westwood[2], Philip Kim[1], Sachdev Sidhu[1], Quaid Morris[1], Craig Smibert[1,3], Howard Lipshitz[1]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Department of Cell and Systems Biology, University of Toronto; 3) Department of Biochemistry, University of Toronto.

RNA-binding proteins (RBPs) interact with *cis*-elements in their target mRNAs to control their splicing, transport, localization, translation, and stability. The *Drosophila* genome encodes about 400 proteins with known RNA-binding domains (RBDs), about three quarters of which are expressed in early embryos, where gene regulation is accomplished exclusively at the post-transcriptional level. Our long-term goal is to identify all RBP-mRNA interactions in early *Drosophila* embryos. To accomplish this, we are using synthetic antibodies, generated by phage display, to perform RNA co-immunoprecipitation (RIP) coupled with microarray-based or next-generation sequencing analysis (RIP-Chip or RIP-seq). After establishing a high-throughput pipeline for synthetic antibody production, we have generated 328 antibodies against 73 RBPs. 18 out of 18 antibodies, representing 8 RBPs, successfully IP their target proteins. We initially focused on three RBPs for genome-wide identification of associated mRNAs: the double-stranded RBP, Staufen (STAU); the PUF protein, Pumilio (PUM); and the TRIM-NHL protein, Brain Tumor (BRAT). Computational analysis of their targets predicted specific RNA structural motifs for STAU binding, and two distinct single-stranded motifs, one for PUM binding and one for BRAT binding. Since the latter is the first predicted binding motif for any TRIM-NHL protein, we confirmed it using an *in vitro* assay known as RNAcompete and showed that it mediates BRAT-directed repression in S2 cells. Unexpectedly, PUM and BRAT were found to associate with largely distinct sets of mRNAs, whose post-transcriptional fates differ. While mRNA targets of both proteins appear to be translationally repressed, the majority of BRAT's target mRNAs are also degraded during the maternal-to-zygotic transition. Indeed, gene expression profiling in *brat* mutants revealed that BRAT is required for the degradation of hundreds of mRNAs in early embryos.

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Targeted degradation of *Gadd45* mRNA by the nonsense-mediated decay pathway is essential for viability. Jonathan O. Nelson, Alex Chapin, Mark M. Metzstein. Department of Human Genetics, University of Utah, Salt Lake City, UT.

Nonsense-mediated mRNA decay (NMD) is a cellular quality control mechanism that selectively degrades nonsense-mutation containing transcripts. In addition to preventing the expression of nonsense mRNAs, NMD also has a significant role regulating normal gene expression, as it degrades many native, non-mutant, mRNAs. In complex organisms such as *Drosophila* and mammals, loss of NMD is lethal, most likely due to over-expression of these native NMD targets. However, it is not known which target(s) are responsible for NMD mutant lethality.

To identify critical NMD targets we performed a screen using deficiencies to suppress the lethality of a semi-viable allele of a core NMD factor, $Upf2^{25G}$. From over 400 DrosDel deficiencies screened, we identified five regions where a heterozygous deficiency significantly increases the percentage of viable animals. In previous work we had identified mRNAs that are direct targets of the NMD pathway at a genome wide level. Of these targets, only one, the stress response factor *Gadd45*, is found within any of the suppressing regions. We have made the first null *Drosophila Gadd45* mutant and find it suppress$Upf2^{25G}$ lethality identically to the deficiency. In addtion, we find that mutants for the obligate downstream signaling partner of *Gadd45*, *Mekk1*, also suppress NMD-mediated lethality.

Our findings reveal that *Gadd45* is a critical NMD target whose over-expression leads to lethality and other defects observed in NMD mutants. This is the first time a direct NMD target responsible for mediating NMD defects has been identified in any animal. Importantly, the mammalian homologues of *Gadd45* are also NMD targets, thus *Gadd45* may be an evolutionarily conserved critical target of NMD-dependent gene regulation.

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Cis-regulation of miRNA clusters tempers strong miRNA phenotypes. Mary Truscott[1*], Abul Bmmk Islam[2], Maxim Frolov[1]. 1) Biochemistry & Molecular Genetics, University of Illinois at Chicago, Chicago, IL; 2) Genetic Engineering & Biotechnology, University of Dhaka, Dhaka, Bangladesh.

The importance of miRNAs in the regulation of fundamental biological processes and disease is well recognized. However, what remains largely unappreciated is that a significant number of miRNAs are embedded within and are often co-expressed with protein-coding gene hosts. Such a configuration raises the possibility of a functional interaction between a miRNA and the gene in which it resides. The two intronic miRNAs embedded in the *dE2f1* gene limit the apoptotic function of dE2f1, but operate in different contexts and act through distinct mechanisms. We previously showed that miR-11 limits the proapoptotic function of dE2F1 in response to DNA damage by repressing cell death genes that are directly regulated by dE2F1, while miR-998 suppresses dE2F1-dependent cell death specifically in *rbf*mutants by elevating EGFR signaling. Strikingly, the deletion of the *mir-11* gene also blocked the expression of pre-mir-998, which suggested that the expression of miR-998 must be tightly regulated. In order to investigate the functional consequences of expression of miR-998 in the absence of *mir-11*, we generated new mutant alleles by Recombinase-Mediated Cassette Exchange. Replacement of the*mir-11* gene with a short hairpin miRNA targeting mCherry, or with a mir-1/998 chimera, rescued miR-998 expression. In the absence of *mir-11*, miR-998 expression lead to strong pleiotropic developmental defects. Therefore, the expression of miR-11 and miR-998 is linked, and miR-11 functions to temper the role of miR-998 in modulating developmental signaling. Furthermore, we propose that the expression of polycistronic miRNA clusters is regulated such that the individual miRNAs are not always expressed at the same levels. This differential expression leads to differences in the modulation of individual miRNA targets, and consequent changes in biological outcome.

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The evolution of Drosophilid piRNA generating clusters is extremely rapid and variable. Gung-wei Chirn[1], Reazur Rahman[1], Yuliya Sytnikova[1], Jessica Matts[1], Cosmas Arnold[2], Alexander Stark[2], Michael Yu[3], Bonnie Berger[3], **Nelson Lau**[1]. 1) Biology, Brandeis University, Waltham,MA, USA; 2) Research Institute of Molecular Pathology, Vienna Biocenter, Austria; 3) Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA USA.

Animals express two forms of Piwi-interacting RNAs (piRNAs) clusters: non-coding intergenic clusters, and genic clusters from the 3'UTR of certain protein-coding transcripts. Although these classes are conserved from flies to mammals, piRNA cluster homologs are difficult to define because intergenic piRNA clusters are filled with rapidly evolving transposon relic sequences, and the evolutionary divergence between flies and mammals is extensive (>470mya). We hypothesize that genic piRNA clusters amongst closely related Drosophilids might better inform on piRNA cluster evolution. After deeply sequencing piRNAs from follicle-cell enriched RNAs from *D.melanogaster (D.mel)*, *D.erecta* (*D.ere*), *D.yakuba* (*D.yak*), and *D.virilis* (*D.vir*), we applied a bioinformatics pipeline that discovered 289 genic piRNA clusters in *D.mel*. Although *D.ere* and *D.yak* are estimated both to have simultaneously diverged from *D.mel*, there was surprisingly a much larger fraction of conserved genic piRNA clusters between*D.mel* and *D.ere* compared to *D.yak and D.vir*. This unexpected difference in genic piRNA cluster conservation is not simply a result of differences in piRNA deep sequencing or gene expression profiles between species. Therefore, we conclude that sequences in genic piRNA transcripts that specify mature piRNA production are evolving more rapidly than protein-coding sequences within these transcripts. This feature may provide better resolution of the divergence between *D.mel*, *D.ere*, and *D.yak*, help define species-specific piRNA clusters, and possibly inform on sequence signals promoting piRNA biogenesis. Finally, by evaluating intergenic piRNA clusters within syntenic blocks, we discovered a difference in conservation between the *flamenco* and *42AB* piRNA clusters. This varying degree of conservation between the two largest intergenic piRNA clusters characterized in *D.mel* is surprising given their links to fecundity and transposon control in the germline.

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Development of high pressure freezing and correlative light/electron microscopy for *Drosophila* larvae identifies novel subcellular lumen intermediates. Linda Nikolova[1,2], **Mark Metzstein**[1]. 1) Dept Human Gen, Univ Utah, Salt Lake City, UT; 2) Electron Microscopy Core Laboratory, Univ Utah, Salt Lake City, UT.

We are interested in the mechanisms by which tracheal terminal cells generate their membrane-bound intracellular lumens during larval development. The luminal membrane is though to form through a process of vesicle trafficking and fusion. However, such vesicle fusion has not yet been directly observed. Transmission electron microscopy (TEM) remains the method of choice for examining cell ultrastructure and it is critical that fixation methods used for TEM analysis maintain membranes in their native states. One such method is high pressure freezing (HPF), in which rapid freezing under high pressures is used to prevent the formation of water ice crystals that otherwise disrupt cellular architecture.

We have now adapted HPF techniques to fix intact larval *Drosophila*. Surprisingly, we have found that subcellular lumen formation does not proceed through direct fusion of free cytoplasmic vesicles, but rather appears to proceed through a multimembrane intermediate composed of vesicles bound within a novel subcellular compartment. Further evidence for this mechanism has come from our analysis of terminal cells mutant for the gene *Rabconnectin-3A (Rbcn-3A)*. To analyze ultrastructure in *Rbcn-3A* terminal cells we have adapted our HPF method such that fluorescence of transgenic markers is maintained during fixation. This so called correlative light /electron microscopy procedure allows us to identify labeled cells in mosaics and then perform TEM on the same samples. We have found that *Rbcn-3A* mutant terminal cells contain complex multi-membranous structures instead of mature lumens. Rbcn-3A is a regulator of the vacular ATPase (V-ATPase), which is required for acidification of subcellular compartments, and we have found reduction of V-ATPase activity in terminal cells phenocopies Rbcn-3A on the ultrastructural level. Our results suggest the formation of a specialized subcompartment precedes subcellular lumen formation, and that subsequent acidification of this compartment is required for resolving these membranes into the mature, subcellular lumen.

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A genome-wide resource for the analysis of gene function and protein localization in *Drosophila*. Mihail Sarov[1], Christiane Barz[2], Katja Finkl[2], Marco Hein[2], Stephan Janosch[1], Nicole Plewka[2], Bettina Stender[2], Dana Suchold[1], Vinay Vikas[3], Matthias Mann[2], Mani Ramaswami[4], K. VijayRaghavan[3], Pavel Tomancak[1], **Frank Schnorrer**[2]. 1) Max Planck Institute of Cell Biology and Genetics, Dresden, Germany; 2) Max Planck Institute of Biochemistry, Martinsried, Germany; 3) National Centre for Biological Sciences, Bangalore, India; 4) Trinity College Dublin, Ireland.

The *Drosophila* genome contains >13,000 protein coding genes. Despite the persisting efforts of the community expression, localisation and function for the majority of these proteins remain poorly investigated. Important reasons include the lack of specific antibodies or reporter constructs to visualise these proteins. Here, we present a genome-wide GFP-tagged genomic fosmid library as resource for the fly community. The fosmid clones contain about 36 kb genomic DNA, comprising the gene and most regulatory information required for its correct expression. Using recombineering in bacteria, we have generated more than 10,000 clones (> 80 % of the genome), in which we tagged each protein at its C-terminus with the multi-functional 2xTY1-sGFP-V5-pre-TEV-BLRP-3xFLAG tag, enabling both live imaging and biochemical purifications. We have created transgenic lines for more than 800 tagged proteins and assessed the functionality of the library by investigating protein expression and localisation patterns in embryos, pupae and adults, both with stainings and *in toto* live imaging approaches. We find that many proteins can be visualised live at endogenous expression levels, making this library a valuable resource for live imaging. Interestingly, a large fraction of proteins localise to subcellular compartments within the investigated tissues of the

intact animal. To test functionality of the tagged proteins we crossed a representative set of tagged clones into genetic null mutants and find that about two thirds are entirely functional, showing the success of our tagging strategy. Finally, we provide proof of principle results demonstrating that our clones allow interaction proteomics from adults flies and developing pupae. Taken together, we envision that this resource will be of significant value for the fly community and will be made publicly available..

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Computational tissue labeling: Tissue and Cellular Recognition in Developing *Drosophila* Embryos. Soile V E Keranen[1], Jonathan T Barron[2], Pablo Arbeláez[2], Mark D Biggin[1], Jitendra Malik[2], David W Knowles[1]. 1) Lawrence Berkeley National Laboratory, Berkeley, CA; 2) University of California Berkeley, Berkeley, CA.

Knowing the dimensions and locations of each cell, which tissues they belong to, and how these vary between embryos are essential for quantitating embryo morphology. A major challenge in building an *in silico* embryo atlas to capture this information is assigning every cell to its correct tissue because it is not possible to label a single embryo with tens of tissue specific labels. To overcome this challenge, we have created a computational technique that uses 3D images of embryos stained to label DNA to detect tissues at cellular resolution without the need for tissue-specific labeling in that image. Training and test sets of DNA-stained embryo images were annotated either by hand or by tissue specific gene expression labels to outline the locations of multiple tissues. The great majority of nuclei in the test set images were assigned to the correct tissue using our method. Thus, we can now assign nuclei to their correct tissue in any number of unannotated embryo images automatically given only a DNA stain. We show that nuclear segmentations algorithms optimized for each tissue separately are more accurate in defining the extent of each nucleus than a generic method for whole embryos, illustrating the importance of assigning nuclei to tissues. By comparing the *in silico* atlases for ~100 embryos, we have quantitated a range of morphological features and their variability. We show that the variation between embryos in the number of nuclei per tissue correlates with tissue volume; that there are shape differences between the left and right versions of salivary glands and anal pads, but not pharyngeal muscles; and that the morphological characteristics of tissues correlate with small changes in developmental age and can be used to accurately assign embryos to an age rank. These results establish that our methods provide novel measures of embryo morphology.

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Model-driven data visualization and quantitative animation of developmental signaling. Bomyi Lim[1], Carmeline Silva[2], Adam Finkelstein[3], Ioannis Kevrekidis[2], Stanislav Shvartsman[1]. 1) Department of Chemical and Biological Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, NJ; 2) Department of Chemical and Biological Engineering and Program in Applied and Computational Mathematics, Princeton University, NJ; 3) Department of Computer Science, Princeton University, NJ.

Some aspects of developmental dynamics, such as tissue morphogenesis, can be monitored in real time, while other features like patterns of enzyme activity must be reconstructed from snapshots of embryos arrested in their development. Establishing a systems-level view of developmental dynamics requires automated approaches for data integration, which should combine longitudinal data from live imaging assays and cross-sectional data from fixed samples. Our goal is to combine information from multiple experimental assays that visualize different aspects of developmental dynamics in different embryos into one stereotypical developmental trajectory. We use dynamics of the Extracellular Signal Regulated Kinase (ERK) activation in the early *Drosophila* embryo as a data-rich platform for establishing such data integration. Previous studies with fixed embryos produced an atlas of ERK activation. This atlas takes the form of nine static, human-drawn schematics of the spatial patterns of ERK activation during the first 12 hours of development. We have used a combination of high-throughput imaging, data mining approaches and computational modeling to convert this atlas into a three-dimensional quantitative movie that best represents the data. We describe two approaches of making such movies. The first approach is based on direct mapping of information from fixed images onto frames of a live imaging dataset of tissue morphogenesis. The second approach relies on a data-based mathematical model of ERK activation, which is then "run in real time" within cells of a live imaging data, providing an additional channel corresponding to a model variable. By testing these approaches on rich dataset of spatiotemporal patterns of chemical and mechanical patterns in the early *Drosophila* embryo, we establish robust data integration and model-based animation strategies that can apply to a wide range of developmental systems.

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High-throughput Investigation of Drosophila Brains via Structure-Based Similarity. Florian Ganglberger[1], Laszlo Tirian[2], Florian Schulze[1], Andrew Straw[2], Katja Bühler[1]. 1) VRVis Research Center, Vienna, Austria; 2) Institute of Molecular Pathology, Vienna, Austria.

Identification of suitable transgenic driver lines for small subsets of Drosophila neurons is critical for understanding the role of single neurons and circuits of interconnected neurons in behavior. Comparing neuronal types in large 3D image datasets is a major challenge that is done typically by browsing through Z-projections with subsequent loading of the original confocal image. Besides its time-consuming nature, it prefers neurons that are not hidden behind other neurons, neurons with bright signal and neurons on images with only a few labeled neurons. Therefore, a high- throughput search in large databases requires automated tools for image retrieval. We use a highly optimized structure based neuron retrieval method. Through sparsification, file compression, downscaling, multithreading and code optimizations we made fast and user-friendly investigation of big datasets possible. We tested the method on the Vienna Tile (VT) collection, a large publicly available registered 3D neuronal database (~11.000 images). We retrieved a large number of images representing several driver lines for all queries tested. Depending on neuronal type, query region (complete neuron, projection path or partial arborization pattern) and quality of query image the method delivered up to 100 % true positive images for

the top hundred hits, and - according to our statistics - delivers 50% of all positive images within the top 0.5-2% of the hits. Moreover, the method showed insensitivity to signal to noise ratio and expression broadness of the images. It performs robustly to biological variability and registration errors, since queries retrieved images with as little as 5% overlap of the queried voxels. After a preprocessing step, queries can be executed in ~20 sec per 1000 images. Besides supporting neuroanatomy and behavior studies the method is capable of delivering sufficient data for system genetic approaches. .

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An automated image analysis tool to track cell divisions during *Drosophila* axis elongation. Michael F.Z. Wang[1], Rodrigo Fernandez-Gonzalez[1,2,3]. 1) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada; 2) Cell and Systems Biology, University of Toronto, Toronto, Canada; 3) Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, Canada.

Axis elongation is a conserved morphogenetic process in which embryos extend their head-to-tail or anterior-posterior axis. In *Drosophila* embryos, axis elongation occurs in an epithelial monolayer known as the germband. During germband extension, cells intercalate along the dorsal-ventral axis causing an anterior-posterior elongation of the tissue. In addition, oriented cell divisions at the posterior end of the germband also contribute to germband extension. Notably, cells on the anterior-ventral germband also divide during the final stages of axis elongation. However, the contribution of these cell divisions to germband extension has not been investigated. We used spinning disk confocal microscopy to image germband extension in *Drosophila* embryos. We developed an automated image analysis tool to track cells and quantify the orientation of cell division based on cell outlines. Briefly, an interactive adaptive threshold is applied to find one point or *seed* per cell in the first image of the time sequence. Seeds are grown using the watershed algorithm to detect cell boundaries and are propagated to subsequent time points in the movies where watershed-based segmentation is iterated. Seed propagation takes into account cell movements by calculating the spatial cross-correlation between consecutive images, a measurement of local signal displacement. Finally, dividing cells are automatically detected based on their increased apical cell area, reduced circularity, and dumbbell morphology. The corresponding parent seed is then split into two seeds to segment daughter cells. The angle of cell division is determined to be the angle between the centroids of the two daughter cells relative to the anterior-posterior axis of the animal. Using this approach, we found that cells on the anterior-ventral germband divide parallel to the anterior-posterior axis of the embryo, possibly contributing to germband extension. .

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FlyVar: a database for genetic variation in Drosophila melanogaster. Rui Chen[1,2], Lichu Jiang[1], Yong Chen[4], Nele Haelterman[3], Hugo Bellen[2,3,5], Fei Wang[4]. 1) HGSC,Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas; 3) Program of Developmental Biology, Baylor College of Medicine, Houston, Texas; 4) Information Processing, Department of Computer Science and Technology, Fudan University, Shanghai, China; 5) Howard Hughes Medical Institute.

With the rapid progress and cost reduction of next generation sequencing technology, direct sequencing is becoming the most rapid and cost effective method for mutation identification. However, analysis and especially interpretation of large amounts of sequencing data could be challenging. FlyVar is a free integrative platform that is designed to specifically address the increasing need of next generation sequencing data analysis in the *Drosophila* research community. It is composed of three parts. First, it has a database containing 5.94 million DNA polymorphisms in *Drosophila melanogaster*, equivalent to an average of one variant per 50 bases across the genome. Modeled after the human 1000 genome project, this data was obtained from whole genome shotgun sequencing of 561 *D. melanogaster*strains or pools of strains. Second, a graphical user interface has been implemented to allow easy and flexible queries of the database. Third, a set of interactive online tools has been developed to enable filtering and annotation of the genomic sequence obtained from individual *D. melanogaster* strains to identify candidate mutations. This tool has been used in analyzing our recent large scale X chromosomal EMS mutagenesis project and yield excellent results. FlyVar enables researchers to analyze the next generation sequencing data for mutation identification without the need of extensive computational training or resources. The tool is available at http://www.iipl.fudan.edu.cn/FlyVar/.

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Identification of novel drug targets for Tuberous Sclerosis Complex by cross-species synthetic screens combining CRISPR-based knockouts with RNAi. Benjamin E. Housden[1], Alexander J. Valvezan[2], Colleen Kelley[1], Richelle Sopko[1], Yanhui Hu[1], Charles Roesel[1], Shuailiang Lin[1], Michael Buckner[1], Rong Tao[1], Bahar Yilmazel[1], Stephanie E. Mohr[1], Brendan D. Manning[2], Norbert Perrimon[1,3]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Genetics and Complex Diseases, Harvard School of Public Health; 3) Howard Hughes Medical Institute.

The tuberous sclerosis complex (TSC) tumor suppressors, TSC1 and TSC2 (gigas), function together in an evolutionarily conserved protein complex that is a point of convergence for major cell signaling pathways regulating mTOR complex 1 (mTORC1). Mutation or aberrant inhibition of the TSC complex is common in a diverse array of human tumor syndromes and cancers across tissue lineages. The discovery of novel therapeutic strategies to selectively target cells with functional loss of this complex is therefore of significant clinical relevance to TSC and sporadic cancers.

We have developed a CRISPR-based method allowing the generation of homogenous mutant *Drosophila* cell lines. By combining *TSC1* and *TSC2* mutant cell lines with RNAi screens against all kinases and phosphatases, we identified synthetic lethal interactions with *TSC1* and *TSC2*. Knockdown of three hits reduced viability of both *Drosophila TSC1* and *TSC2* mutant cells but left wild-type cells unaffected. Importantly, knockdown of all three genes displayed similar selective viability effects in mammalian TSC2-deficient

cell lines, including human tumor-derived cells, illustrating the power of this cross species screening strategy to identify new drug targets.

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Cooperation of Mad and Akt signaling in a *Drosophila* model of epithelial plasticity. Courtney Onodera[4,5], Björn Gärtner[6], Katrina S. Gold[2,5], Samantha Aguinaldo-Wetterholm[2,5], David Casso[2,5], J. Alex Rondon[2,5,7], Yoko Katsuno[2,5], Samuel Meier[6], Aiguo Tian[2,5,8], Rik Derynck[1,2,3,5], Jun S. Song[4,5,9], Julia Zeitlinger[6], **Katja Brückner**[1,2,3,5]. 1) Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research; 2) Department of Cell and Tissue Biology; 3) Cardiovascular Research Institute; 4) Institute for Human Genetics; 5) University of California San Francisco, CA; 6) Stowers Institute for Medical Research, Kansas City, MO; 7) present address: Genentech; 8) present address: UT Southwestern; 9) present address: University of Illinois at Urbana-Champaign.

Transforming Growth Factor-β (TGF-β) or the related Bone Morphogenetic Proteins (BMPs) cooperate with Akt signaling in epithelial plasticity and epithelial-to-mesenchymal transition (EMT) during development and in pathologies such as fibrosis and tumor metastasis. However, the molecular basis of this cooperation remains incompletely understood. *Drosophila* has been an excellent model to study epithelial architecture and plasticity in vivo, yet no cell-based system has been available for the molecular dissection of epithelial plasticity. We now introduce KaBrü1D, a *Drosophila* epithelial cell line closely related to wing imaginal disc cells, that undergoes BMP/decapentaplegic (dpp) induced epithelial plasticity, similar to the elongation of wing imaginal cells during thorax closure. Based on an RNAi screen comprising all kinases and phosphatases, expression profiling, and ChIP analyses, we identified Mad (mothers against dpp) transcriptional targets and genes functionally involved in Dpp/BMP-induced epithelial plasticity. Akt/Tor signaling is essential in this process, and activity of this pathway is enhanced over the course of several days of BMP stimulation, consistent with a secondary transcriptional wave leading to elevated receptor tyrosine kinase signaling. We investigated the mechanism of cooperation between the Dpp and Akt pathways, focusing on the surprising finding that lack of Akt/Tor signaling results in increased Mad binding, yet in the reduced expression of many Mad target genes. Using bioinformatics analysis, we identified associated transcription factor motifs in differentially expressed Mad targets and we dissect this regulation in cell culture and during thorax closure in vivo.

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Cis-interactions between Notch and its ligands block ligand-independent Notch activity. William Palmer, Dongyu Jia, **Wu-Min Deng**. Dept Biological Sci, Florida State Univ, Tallahassee, FL.

The Notch pathway is integrated into numerous developmental processes and therefore is fine-tuned on many levels, including receptor production, endocytosis, and degradation. Notch is further characterized by a two-fold relationship with its Delta-Serrate (DSL) ligands, as ligands from opposing cells (*trans*-ligands) activate Notch, but ligands expressed in the same cell (*cis*-ligands) inhibit signaling. We show that cells without both cis and trans ligands are able to mediate Notch-dependent developmental events during *Drosophila* oogenesis, indicating ligand-independent Notch activity occurs when the receptor is free of *cis* and *trans* ligands. Furthermore, *cis*-ligands can reduce Notch activity in endogenous and genetically-induced situations of elevated *trans*-ligand-independent Notch signaling. We conclude that *cis*-expressed ligands exert their repressive effect on Notch signaling in cases of *trans*-ligand independent activation, and propose a new function of *cis*-inhibition which buffers cells against accidental Notch activity.

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***Drosophila* Matrix Metalloproteinase 2 mediates long-distance attenuation of follicle stem cell proliferation by cleaving Dlp to inhibit Wg diffusion. Xiaoxi Wang**[1,2], Andrea Page-McCaw[1,2,3]. 1) Development of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN; 2) Program in Developmental Biology, Vanderbilt University Medical Center, Nashville, TN; 3) Development of Cancer Biology, Vanderbilt University Medical Center, Nashville, TN.

Wnt ligands travel in the extracellular space over many cell diameters to act on distant cells. It remains unclear how this spreading is regulated, and its functional significance has recently been challenged. In the *Drosophila* ovary, Wingless (Wg) promotes the proliferation of follicle stem cells. Wg is produced by apical cells located ~50μm or 5 cell diameters away and forms an extracellular gradient towards FSCs. We find in addition to Wg, apical cells also produce a negative regulator of Wg spreading, matrix metalloproteinase 2 (Mmp2). *Mmp2*-deficient ovaries displayed increased Wg distribution, activity, and stem-cell proliferation, which was suppressed by reducing *wg* or *armadillo*. Mmp2 limits Wg spreading by antagonizing the functions of Dally-like protein (Dlp). Dlp is a glypican that binds Wg and promotes its long-range diffusion in the wing disc, and we show that it functions similarly in the ovary. In the ovary, *Mmp2* mutants exhibited changes in Dlp distribution, and reducing *dlp* attenuates *Mmp2*loss-of-function phenotypes. In cell culture, Mmp2 cleaves Dlp in the N-terminal subunit, induces Dlp re-localization away from the plasma membrane to intracellular sites, and thus abolishes the cell-surface interaction between Dlp and Wg. We are investigating Dlp proteolysis *in vivo* by constructing a non-cleavable Dlp and testing how it modifies Wg signaling. We are also testing if this novel interaction between an MMP and a glypican applies to the regulation of other signaling pathways and if it has conserved mammalian counterparts.

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Ion channel function regulates Dpp release to correctly specify pattern. Emily Bates, Giri Dahal, Sarala Pradhan, Colleen Bartman. Department of Pediatrics, University of Colorado Denver, Aurora, CO.

Loss of ion channel function during embryogenesis can cause craniofacial and limb abnormalities in mammals, but the underlying reason for these defects has remained elusive. Here, we show that depolarization of cells of the larval wing disc govern a novel mechanism of morphogenesis. Loss of the Irk2 potassium channels leads to wing patterning phenotypes. We find that the Irk2

potassium channel is required for pulsatile release of Dpp/BMP in the wing disc, and that the inwardly rectifying conductivity of Irk2 is required for this function. In neurons, inward rectification enables recovery from an action potential that is necessary for the next round of neurotransmitter vesicle release. We hypothesized that a similar mechanism regulates Dpp/BMP release from undifferentiated cells. We found that Irk2 mutant cells remain depolarized and release excessive Dpp/BMP, potentially prematurely depleting Dpp/BMP stores. Our results lead to a new model of controlled Dpp/BMP release based on the activity of ion channels.

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Secretion and exovesicle mediated transport of the Hedgehog morphogen is regulated by the ESCRT complex. T. Matusek, F. Wendler, M. Fürthauer, S. Pizette, G. D'Angelo, P. Thérond. Institute of Biology Valrose, UNS - CNRS UMR7277 - Inserm U1091, Faculté des Sciences, Parc Valrose, 06108, Nice cedex 2, France.

The Hedgehog morphogen plays an instructive role in various developmental processes. Malfunction of both Hh signaling and secretion leads to severe developmental disorders from fly to human. Although the molecular pathway of Hh signaling in the receiving tissue is quite well investigated, how this dually lipidated, highly hydrophobic molecule is secreted and travels from the producing cells in the hydrophilic extracellular environment is still poorly understood. Current models for secretion and transport include Hh multimerisation, cytoneme-mediated transport, binding to lipoprotein particles. However the possibility of Hh transport via membranous vesicles released by cells (also called exovesicles) has never been functionally explored.
Following a proteomic analysis of proteins present on exovesicle-like structures from conditioned medium of Hh-expressing cells *in vitro*, we have analyzed a set of candidate proteins including members of the ESCRT machinery focusing primarily on the Hh producing tissue *in vivo*. We provide evidence that Hh secretion is dependent on ESCRT function both in gain-of-function and in loss-of function conditions. We show, that interfering with ESCRT activity in Hh-producing cells *in vivo* leads to the retention of Hh at the external surface of producing cells and a specific loss of Hh target expression. We have also found Hh and ESCRTs at certain distance from their source of production, spreading together in common particles. Moreover we were able to trap Hh and ESCRT containing paticles at the external surface of recipient cells expressing the Hh receptor.
Our findings reveal a novel function for ESCRT proteins in controlling morphogen activity. They also provide evidence for a previously unidentified mechanism for Hh release and intercellular communication.

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Nanotubes mediate the niche-stem cell signaling in *Drosophila* testis. M. Inaba[1,2,3], M. Buszczak[2], YM. Yamashita[1,3]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 3) Howard Hughes Medical Institute, University of Michigan Ann Arbor, MI.

Stem cell niches provide resident stem cells with signals that specify their identity. The niche signals are believed to be short-range in nature such that only stem cells but not their differentiating progeny receive the self-renewing signals. However, the cellular mechanisms that limit niche signaling to stem cells remain poorly understood. Here we show that the *Drosophila* male germline stem cells (GSCs) form previously unrecognized structures, microtubule-based (MT)-nanotubes, which extend into the hub, a major niche component. We show that MT-nanotube formation requires IFT (intraflagellar transport) proteins, and that MT-nanotubes mediate Dpp signaling. Perturbation of MT-nanotubes compromises activation of Dpp signaling within GSCs, leading to GSC loss. Moreover, Dpp ligand and receptor interaction is necessary and sufficient for inducing MT-nanotube formation. We propose that MT-nanotubes function to restrict niche-stem cell signaling by providing exclusive surface area for efficient receptor-ligand interaction, contributing to the short-range nature of the niche-stem cell signaling.

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***Rme-8* depletion perturbs Notch recycling and predisposes to pathogenic signalling. Maria J. Gomez-Lamarca**[1], Laura A. Snowdon[1], Ekatarina Seib[2], Thomas Klein[2], Sarah J. Bray[1]. 1) Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom; 2) Institute of Genetics, Heinrich-Heine-University Düsseldorf, Universitätsstr. 1 40225 Düsseldorf, Germany.

Notch signalling is a major regulator of cell fate, proliferation and differentiation. Like many other signalling pathways its activity is strongly influenced by intracellular trafficking. Besides contributing to signal activation and down-regulation, differential fluxes between trafficking routes can be conducive to aberrant Notch pathway activation. Investigating the function of the retromer-associated DNAJ protein Rme-8, *in vivo,* we demonstrate a critical role in regulating Notch receptor recycling. In the absence of Rme-8, Notch accumulated in enlarged tubulated Rab4-positive endosomes and, as a consequence, signalling was compromised. Strikingly, when the retromer component *Vps26* was depleted at the same time, Notch no longer accumulated and instead was ectopically activated. Likewise, depletion of ESCRT-0 components *Hrs* or *Stam* in combination with *Rme-8* also led to high levels of ectopic Notch activity. Together these results highlight the importance of Rme-8 in co-ordinating the normal endocytic recycling route and reveal that its absence predisposes towards conditions where pathological Notch signalling can occur.

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Human and fly genetics implicate a *CD2AP/cindr* susceptibility network at synapses in Alzheimer's disease. Nikolaos Giagtzoglou[1,2], Paula Porter[1,2], Kathleen Quast[1,2], Benjamin Arenkiel[1,2],**Joshua Shulman**[1,2]. 1) Baylor College of Medicine, Houston, TX; 2) Duncan Neurological Research Institute, Houston, TX.

Genomewide scans have identified 22 loci associated with Alzheimer's disease (AD) risk. Based on a functional validation strategy

examining gene candidates in *Drosophila*, we implicate a susceptibility network comprised of *CD2AP* and related mediators of adhesion (*CASS4*, *FERMT2, PTK2B*) and endocytosis (*PICALM*, *BIN1*, *AP-2α, RIN3*). Genetic manipulation of fly homologs for many of these loci alters the neurotoxicity of Tau, which forms neurofibrillary tangle pathology in AD. In order to better define the nervous system function of this putative regulatory pathway, we have studied *cindr*, the single conserved fly homolog of the *CD2AP* SH3-domain adaptor protein. Cindr is highly expressed in neurons, showing enrichment at both peripheral and central synapses. Immunoprecipitation of Cindr from adult brains confirms an interaction with Actin and identifies novel associations with synaptic proteins, including the Ca^{2+} sensor, Synaptotagmin, the synaptic vesicle reserve pool marker, Synapsin, and multiple core mediators of clathrin-dependent endocytosis, including AP-2σ, EPS-15, and Hsc-70, which regulate synaptic vesicle recycling. We have also generated a *cindr* deletional null allele through transposable element-mediated recombination. Since global development and maintenance of the brain appeared normal, we explored a synaptic regulatory role. Studies at the larval neuromuscular junction reveal a "ghost bouton" phenotype, consistent with pre- and post-synaptic membrane uncoupling. Further, electrophysiologic studies show preserved basal neurotransmission but increased facilitation following high-frequency stimulation, consistent with altered synaptic vesicle release probability and impaired plasticity. We are currently extending our studies to mouse models, beginning with confirmation of the CD2AP synaptic localization and examination of hippocampal neurophysiology in knockout animals. Based on our results, we postulate that dysfunction within the CD2AP susceptibility network attenuates synaptic efficacy, and leads to enhanced vulnerability to Tau-induced neuronal injury in AD. .

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The PINK1/Parkin Pathway Regulates the Mitochondrial Outer Membrane Localization and Translation of Select Nuclear-Encoded Respiratory Chain Component mRNAs. Stephan Gehrke[1], **Zhihao Wu**[1], Michael Klinkenberg[2], Georg Auburger[2], Su Guo[3], Bingwei Lu[1]. 1) Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA; 2) Experimental Neurology, Goethe University Medical School, Theodor Stern Kai 7, 60590 Frankfurt am Main, Germany; 3) Department of Biopharmaceutical Sciences, Programs in Biological Sciences and Human Genetics, University of California, San Francisco, CA 94143, USA.

Mitochondria play essential roles in many aspects of biology, with mitochondrial dysfunction having been linked to numerous diseases. Central to mitochondrial function is oxidative phosphorylation, accomplished by respiratory chain complexes (RCCs) encoded by nuclear and mitochondrial genomes. How RCC biogenesis is regulated in metazoans is poorly understood. We show that PINK1 and Parkin, two Parkinson's disease-associated genes, direct localized translation of certain nuclear-encoded *RCC (nRCC)* mRNAs. Translationally repressed *nRCC* mRNAs are localized in a PINK1/Tom20-dependent manner to mitochondrial outer membrane, where they are translationally de-repressed and activated by PINK1/Parkin through the displacement of translation repressors, including Pumilio and hnRNP-F/H, through a Parkin mediated ubiquitination, and the binding of activators such as eIF4G. Inhibiting the translation repressors rescued *nRCC* mRNA translation and neuromuscular-degeneration phenotypes of *PINK1* mutant, whereas inhibiting eIF4G had opposite effects. Our results reveal conserved functions of the PINK1/Parkin pathway in mRNA physiology and suggest new strategies to restore diseased mitochondria.

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The ALS/FTD *C9ORF72* hexanucleotide expansion disrupts nucleocytoplasmic transport via RanGAP1. Ke Zhang[1], Christopher Donnelly[2], Aaron Haeusler[3], Rita Sattler[2], Jiou Wang[3], Jeffrey Rothstein[1,2,4,5], Thomas Lloyd[1,4]. 1) Department of Neurology, Johns Hopkins University, Baltimore, MD. 21205; 2) Brain Science Institute, Johns Hopkins University, Baltimore, MD. 21205; 3) Department of Biochemistry and Molecular Biology, Johns Hopkins University, Baltimore, MD. 21205; 4) The Solomon H. Snyder Department of Neuroscience,Johns Hopkins University, Baltimore, MD. 21205; 5) Department of Cellular and Molecular Medicine, Johns Hopkins University, Baltimore, MD. 21205.

A GGGGCC hexanucleotide repeat expansion (HRE) in *C9orf72* is the most common genetic cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), with its underlying mechanism poorly defined. A candidate-based, forward genetic screen in *Drosophila* identified RanGAP, a key regulator of nucleocytoplasmic (NT) transport, as a potent suppressor of HRE-mediated neurodegeneration. We show that other proteins of the NT machinery also genetically interact with HRE. Moreover, RanGAP physically interacts with HRE and is mislocalized in *Drosophila*, neurons differentiated from induced pluoripotent stem cells (iPSNs) from *C9orf72* patients, and *C9orf72* patient brain tissue. Both *Drosophila* models and *C9orf72* ALS iPSNs display NT defects that can be rescued by small molecules targeting the HRE or the NT machinery. Consequently, a nuclear protein, TDP-43, is mislocalized to the cytoplasm, and our genetic data suggest this leads to toxicity, as TDP-43 loss-of-function suppresses HRE-mediated neurodegeneration. Our data identify a novel pathway underlying the pathogenesis of ALS/FTD and provide a core target for disease pathogenesis.

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Inter-cellular transmission of huntingtin aggregates in the *Drosophila* central nervous system. Margaret Pearce[1], Ellen Spartz[1], Weizhe Hong[1,2], Liqun Luo[1,2], Ron Kopito[1]. 1) Stanford University, Stanford, CA; 2) Howard Hughes Medical Institute.

Huntington's disease (HD) is a neurodegenerative disorder caused by a dominantly inherited mutation that results in the expansion of a polyglutamine (polyQ) stretch located near the N-terminus of the protein huntingtin (Htt). Expansion of this polyQ stretch beyond a threshold of 37 glutamines causes Htt to become aggregation-prone and form intracellular inclusion bodies that can be detected in the brains of HD patients. Emerging evidence supports the hypothesis that Htt aggregates have prion-like properties—aggregates present in the extracellular space or in neighboring cells can convert wild-type Htt expressed in the cytoplasm of cultured mammalian cells from its normally soluble state to an aggregated form. To investigate how cell-to-cell spread of Htt aggregation could occur within the

defined constraints of an intact tissue, we have established a *Drosophila* model in which mutant Htt and wild-type Htt are expressed in two independent cell populations within the same brain. Using this model, we have demonstrated that mutant Htt aggregates can be transferred from pre-synaptic neurons into the cytoplasm of neighboring glia, where the aggregates nucleate the aggregation of wild-type Htt proteins. Intriguingly, transfer of Htt aggregates from neurons to glia requires several components of the glial phagocytic machinery, suggesting that neuronal Htt aggregates initially enter glia through a phagocytic pathway but then escape from the phagolysosomal compartment to gain access to wild-type Htt in the cytoplasm. These data demonstrate that inter-cellular propagation of Htt aggregates indeed occurs in an intact central nervous system and point to an unexpected role for phagocytic glia in the spread of HD neuropathology.

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Neuronal mitochondrial defects elevate synthesis of lipid droplets in glia and promote neurodegeneration. Lucy Liu[1], Ke Zhang[2], Hector Sandoval[3], Shinya Yamamoto[3,4,5], Manish Jaiswal[3,6], Elisenda Sanz[7], Zhihong Li[3], Jessica Hui[7], Brett Graham[3], Albert Quintana[7,8,9], Hugo Bellen[1,2,3,4,5,6]. 1) Department of Neuroscience, Baylor College of Medicine; 2) Structural and Computational Biology & Molecular Biophysics Graduate Program, Baylor College of Medicine; 3) Department of Molecular and Human Genetics, Baylor College of Medicine; 4) Program in Developmental Biology, Baylor College of Medicine; 5) Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital; 6) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030; 7) Center for Integrative Brain Research, Seattle Children's Research Institute; 8) Center for Developmental Therapeutics,; 9) Department of Pediatrics, University of Washington, Seattle, WA, 98195.

An increase in lipid droplets (LD) has been implicated in some metabolic disorders but their role in neurodegeneration is ill defined. Through an unbiased forward genetic screen to uncover genes that lead to neurodegeneration in photoreceptors (Yamamoto and Jaiswal et al., 2014, Cell), we identified various nuclear encoded genes that affect mitochondrial function, including fusion, translation, and complex function. Mutations in these genes lead to a transient but severe accumulation of LD in the glia prior to the onset of neurodegeneration. These mutants exhibit increased levels of reactive oxygen species (ROS), which promote c-Jun-N-terminal Kinase (JNK) and Sterol Regulatory Element Binding Protein (SREBP) activity in neurons, leading to LD accumulation in glia, which are peroxidated and cause neurodegeneration. However, this can be significantly delayed with the cell specific reduction of ROS, JNK or SREBP, or by overexpressing lipases. Importantly, a similar pathway leads to glial LD accumulation in *Ndufs4*$^{-/-}$ mice, suggesting that this process is conserved. Furthermore, a brief administration of antioxidants to mutant flies and mice significantly delays neurodegeneration. In summary, we show a novel model for the demise of neurons based on increased ROS in neurons that leads to glial LD accumulation (Liu et al, 2014, Cell). We propose that the evolutionarily conserved synergism between ROS and LD may be an important biomarker and accelerator of neurodegenerative disease.

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The Bubblegum model of Adrenoleukodystrophy provides a basis for new therapeutic approaches. Hannah B Gordon, Anthea Letsou. Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT.

Altered lipid metabolism is a recognized contributor to neurodegenerative diseases such as Adrenoleukodystrophy (ALD). For most of these diseases, therapeutic options are currently limited. Significant therapeutic progress might be expected with the development of animal models. Two Drosophila mutants which fail to activate long- and very long-chain fatty acids (FAs) were used to probe the relationship between lipid homeostasis and nervous system function. Adults homozygous for mutations in *bubblegum* (*bgm*) or *double bubble* (*dbb*) (encoding acyl CoA-synthetases, ACS's) share age- dependent neurodegeneration (ND) and exhibit increased very long chain FA levels, a distinguishing feature of ALD. Thus, ND could be due to precursor accumulation and/or product lack. Here, we demonstrate that dietary supplementation with medium chain FA rescues ND in mutants and that manipulations of light/dark cycles (designed to increase the demand for product while leaving an accumulation of precursor unchanged) enhance ND in mutants. Together, these results demonstrate that a lack of activated FA product is causative of ND in *bgm* and *dbb* mutants. Furthermore, these data support observations that therapies designed to reduce toxic accumulations of very long chain FAs in patients with ALD are ineffective and suggest an alternate approach to therapy, dependent upon providing the missing product(s). In extending our studies to humans, we have identified a leukodystrophy patient and his unaffected brother who both harbor a rare mutation in a human homolog of *bgm*. The affected proband also suffers from epilepsy. Based on our fly studies, we suggest that stress, by use or by trauma, precipitates ND in an ACS mutant background. Our studies provide evidence for long-hypothesized gene-gene and gene- environment interactions proposed as an explanation for lack of shared ALD phenotypes in siblings harboring identical mutations. Our identification of new genes (ACSs), mechanisms (lack of product), and environments (trauma, seizures) that contribute to ALD leaves us well poised to pursue therapeutic options for the treatment of ALD and related neurometabolic diseases. .

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Mutations in SLC25A, a mitochondrial carrier protein, protect against systemic manganese-induced neuronal toxicity. Jan R Slabbaert[1,2], Sabine Kuenen[1,2], Ana Clara Fernandes[1,2], Jef Swerts[1,2], Valerie Uytterhoeven[1,2], Jaroslaw Kasprowicz[1,2], Ronny Blust[3], Patrik Verstreken[1,2]. 1) Center for the Biology of Disease, VIB, Leuven, Vlaams-Brabant, Belgium; 2) Center for Human Genetics and Leuven Research Institute for Neurodegenerative Diseases (LIND), KULeuven, Leuven, Vlaams-Brabant, Belgium; 3) Department of Biology, University of Antwerp, Antwerp, Belgium.

Homeostatic regulation of manganese (Mn) is essential for the nervous system. This feature is underscored by the neurological symptoms such as movement disorders and Parkinson-like phenotypes caused by manganese toxicity; however, processes that mediate

neuronal intracellular Mn homeostasis under physiological and pathological conditions are poorly understood. In a genetic screen in*Drosophila*, designed to identify genes involved in synaptic function, we identified an evolutionary conserved mitochondrial carrier protein of the SLC25A family. Loss of function mutants exhibit excess of cytoplasmic and mitochondrial Mn, providing a genetic tool to investigate Mn handling in neurons. In *Drosophila*, SLC25A localizes to mitochondria and complete loss of *slc25a* results in dysfunctional and swollen mitochondria, accumulations of reactive oxygen species followed by progressive neurotoxicity and defects in synaptic transmission. While complete loss of the protein results in severe mitochondrial defects, heterozygous loss of *slc25a* completely rescues Mn-induced death and toxicity. Our data are consistent with a model where SLC25A is a mitochondrial gatekeeper of intracellular Mn homeostasis, a function central to neuronal function and survival. In addition, our work also suggests that targeting mitochondrial Mn-buffering may be protective to Mn-toxicity. .

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Early zygotic dosage compensation in *Drosophila melanogaster* is *Sxl* dependent. Susan E. Lott[1,2], Jacqueline E. Villalta[3], Michael B. Eisen[2,3]. 1) Evolution and Ecology, University of California, Davis, Davis, CA; 2) Department of Molecular and Cell Biology, University of California, Berkeley, CA; 3) Howard Hughes Medical Institute, University of California, Berkeley, CA.

There is a temporal gap between the beginning of zygotic transcription in early embryonic development and the onset of the well-characterized MSL-mediated dosage compensation mechanism. In a previous study using single embryo RNA-Seq to characterize transcript levels in female and male embryos, we found many genes to be dosage compensated before the onset of MSL-mediated dosage compensation. Hence, another mechanism for compensation must be active in early embryogenesis. Earlier observations had suggested a direct role for the sex determination master switch gene *Sex lethal*(*Sxl*) in early compensation. Gergen and Cline showed that early embryonic differences in *runt* dose in females are functionally compensated in an MSL-independent, SXL-dependent manner. Also, the 3' UTRs of X chromosome derived genes are strongly enriched for predicted Sxl binding sites. To investigate the role of *Sxl* in early embryonic dosage compensation, we profiled expression in female and male embryos with 0,1, and 2 copies of *Sxl*, over four embryonic stages. We find that *Sxl* mutant females have higher transcript levels than Sxl^{+} females for zygotic genes on the X chromosome, with nearly twice as much transcript as in males. This difference is not likely due to inappropriate MSL-mediated X chromosome hyperactivation in *Sxl* mutant females at this early time, as it would require the establishment of a MSL mechanism far earlier than it is observed in males. We are performing a similar experiment with *Sxl msl*-2 double mutant females to control for this possibility. In all, this work demonstrates that the lower transcript levels in *Sxl*+ females is due to the presence of SXL, thus early zygotic dosage compensation in *D. melanogaster* is SXL-dependent and MSL-independent.

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Low affinity binding site clusters confer Hox specificity and regulatory robustness. Justin Crocker[1], Namiko Abe[2], Lucrezia Rinaldi[2], Alistair P. McGregor[3], Nicolás Frankel[4], Shu Wang[5], Ahmad Alsawadi[6,7], Philippe Valenti[6,7], Serge Plaza[6,7], François Payre[6,7], Richard S. Mann[2], David L. Stern[1]. 1) HHMI Janelia, Ashburn, VA; 2) Columbia University Medical Center; 3) Oxford Brookes University; 4) Universidad de Buenos Aires; 5) New Jersey Neuroscience Institute; 6) Centre de Biologie du Développement; 7) CNRS.

In animals, Hox transcription factors define regional identity in distinct anatomical domains. How Hox genes encode this specificity is a paradox, because different Hox proteins bind with high affinity *in vitro* to similar DNA sequences. Here we demonstrate that the Hox protein Ultrabithorax (Ubx) in complex with its cofactor Extradenticle (Exd) bound specifically to clusters of very low affinity sites in enhancers of the *shavenbaby* gene of *Drosophila*. These low affinity sites conferred specificity for Ubx binding *in vivo*, but multiple clustered sites were required for robust expression when embryos developed in variable environments. Although most individual Ubx binding sites are not evolutionarily conserved, the overall enhancer architecture—clusters of low affinity binding sites—is maintained and required for enhancer function. Natural selection therefore works at the level of the enhancer, requiring a particular density of low affinity Ubx sites to confer both specific and robust expression.

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A cell type specific transcriptional repressor directs selective upregulation of terminal differentiation program. Jongmin Kim, Margaret Fuller. Stanford University, Stanford, CA.

A critical regulatory point in adult stem cell lineages is the switch from proliferation of transit amplifying (TA) progenitors to onset of terminal differentiation. Key to proper differentiation is ability to turn on lineage specific gene expression programs while maintaining transcriptional silence of genes expressed in other cell types. By synchronous *in vivo* differentiation of TA cells in the male germ line stem cell lineage of *Drosophila*, we identified the first new transcripts upregulated as TA cells switch from mitotically dividing spermatogonia to differentiating spermatocytes. Functional analysis of the 43 earliest transcripts identified *tZnF*, encoding a 6 C2H2-type zinc finger protein expressed specifically in male germ cells starting in early spermatocytes and localized to chromatin. Loss of tZnF by knock down as well as CRISPR-Cas9 mediated knock out resulted in strong defects in differentiation and *tZnF-/-* spermatids failed to elongate to make functional sperm. Analysis of transcripts in *tZnF* knock down testes showed dramatic upregulation of over 500 genes normally expressed in specific somatic cell types or organs but not in testis. Surprisingly, for spermatocytes in which the repressor (tZnF) was knocked down, testis Meiotic Arrest Complex (tMAC), the transcriptional activator of spermatocyte-specific genes was responsible for the misexpression of somatic genes. Thus through cell-type specific expression of a transcriptional repressor, tZnF, the developmental program prevents collateral damage expression of somatic specific transcripts inappropriate for the lineage as the genome becomes open for expression of the germ line terminal differentiation program. Our findings highlight the importance of cell type and gene selective transcriptional repression mechanisms for proper differentiation in stem cell lineages.

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Genome-wide futile cycling by Hairy transcriptional repressor reveals mechanism for development of nascent gene regulatory networks. Kurtulus Kok[2], Ahmet Ay[3], **David Arnosti**[1,2]. 1) Dept Biochem & Molec Biol, Michigan State Univ, East Lansing, MI; 2) Program in Genetics, Michigan State University, East Lansing, MI; 3) Departments of Biology and Mathematics, Colgate University, Hamilton NY.

Metazoan transcriptional repressors employ multiple pathways to silence genes, including direct interactions with transcriptional activators and induction of chromatin modifications. For mechanistic understanding of this process, the Drosophila embryo features diverse types of spatially-localized repressors critical for driving gene regulatory transitions. From general epigenetic surveys, it is difficult to directly ascribe these complex effects to individual transcription factors. We analyzed repression induced by the conserved HES family protein Hairy to identify genome-wide effects of this long-range repressor, including chromatin states, transcripts, and RNA polymerase of embryos engineered to express wild-type and mutant forms. Hairy directly coordinates histone acetylation and methylation transitions over wide tracts of the genome, however the repressor showed extensive context-specific responses on targets. Most strikingly, Hairy exhibited biochemical activity on many loci that appear to be unrelated to gene regulation at this stage; extensive chromatin modifications were observed on genes whose transcription was unperturbed, and inactive genes were also subject to significant chromatin modifications. Rather than representing inert binding sites, many regions targeted by Hairy are subject to "futile cycles" of chromatin remodeling that may lay the groundwork for evolution of novel transcriptional connections. We believe that Hairy is not unique in this respect, thus it will be imperative to reconsider the evolutionary implications of "background" interactions between metazoan transcriptional regulators and the genome.

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Color vision: Single base differences in a shared *cis*-regulatory element are critical for *rhodopsin* expression in distinct photoreceptor subtypes. Jens Rister, Claude Desplan. Department of Biology, New York University, New York City, NY.

The nervous system consists of a variety of neuronal cell types that exhibit a remarkable morphological and functional diversity, which is the result of differential gene expression. Some genes are expressed in all neurons, whereas others are restricted to subsets of neuronal cell types. For instance, sensory receptor neurons usually express only one (or very few) sensory receptor gene(s) from a larger repertoire. This generates subsets of sensory receptor neurons with specific response properties. In contrast, downstream signal transduction components are broadly expressed in all sensory receptor neurons of a given modality. To decipher the mechanisms underlying this differential expression of terminal differentiation genes, we analyzed the *cis*-regulatory regions of restricted *rhodopsins* and broadly expressed phototransduction genes.

Both sets of genes share an essential 11 base pair (bp) activator motif in their proximal promoters that is conserved for more than 60 million years of evolution. Interestingly, distinct single bp differences are found in each of the motifs of *rhodopsin* genes (RCSI) that are restricted to subsets of photoreceptors. We show that multimerized motifs that are present in broadly expressed photoreceptor genes are sufficient to drive broad reporter expression in all photoreceptors. In contrast, multimerization of specific RCSI motifs drives expression in distinct subsets of photoreceptors. By changing single bps in RCSI motifs in the context of minimal promoters, we show that subtle bp differences control the expression in specific photoreceptor subsets by weakening activator sites to balance activation and repression, or by generating novel repressor or activator sites. These sites that are often repeated in upstream sequences and act in combination with RCSI to provide subtype-specificity.

In conclusion, our analysis reveals that modifying single bps in a shared *cis*-regulatory element is an efficient evolutionary driving force for the generation of novel neuronal subtypes that ultimately allows the establishment of new subtypes of sensory neurons, a prerequisite for discriminating a broad spectrum of environmental stimuli.

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Integration of repressive and patterning inputs at cardiac gene loci. Jemma Webber, Ilaria Rebay. Ben May Dept Cancer Res, University of Chicago, Chicago, IL.

Development of the heart requires precise implementation of gene expression programs in response to complex upstream signaling inputs. The ETS repressor Yan functions as part of a conserved network downstream of receptor tyrosine kinase (RTK) signaling. The opposing action of Yan and the activator Pointed (Pnt) at several pattern-generating enhancers has invoked a bistable switch model to explain how pathway activation can drive differentiation by shifting from a high-Yan/low-Pnt to a low-Yan/high-Pnt state. For example, at the onset of heart formation, Yan is present in the cardiogenic mesoderm where it acts in opposition to Pnt to regulate expression of the heart identity gene *even-skipped* (*eve*). We have shown that in addition to binding the pattern-generating muscle-heart enhancer (MHE), which drives expression in cardiac precursors in the embryonic mesoderm, Yan occupies two repressive elements that we named D1 and D2. Deletion of the D1 or D2 compromises Yan-mediated repression, leading to elevated *eve* expression and impaired cardiac function. Surprisingly, we find that the Yan-bound D1 and D2 repressive elements also recruit Pnt, a finding not fully explained by the current model. We suggest that Yan/Pnt co-occupancy at the D1 and D2 may both prevent premature activation of *eve* expression by Pnt and limit the strength of Yan repression. In this way the MHE would be poised to respond rapidly to RTK signals once they rise above certain thresholds. We suggest that this may be a widely utilized mechanism to control expression of developmentally important genes. Consistent with this idea, we have shown that Yan and Pnt are broadly co-expressed during development, including in the cardiogenic mesoderm. However, *yan* and *pnt* mutant phenotypes are not consistent with RTK controlled switch-like behavior in all contexts where Yan and Pnt are co-expressed. We instead suggest that whereas cardiogenic expression of *eve* relies on integration of Yan repressive inputs with a Yan/Pnt regulated switch, at other core cardiac network genes such as *tinman*, *pannier* and *midline*, Yan

repression may be integrated with patterning elements activated by transcription factors acting downstream of Dpp, Wg and Hh signaling pathways.

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Differential binding and activation of enhancers by Bcd and Otd in the embryo. Rhea Datta[1], Danyang Yu[2], Stephen Small[1]. 1) Department of Biology, New York University, New York, NY; 2) Fairleigh Dickinson University, 1000 River Road, Teaneck NJ 07666.

Anterior patterning of the Drosophila embryo is largely regulated by the Bicoid (Bcd) protein gradient. Bcd recognizes and binds to a TAATCC motif in target enhancers through a lysine (K) at position 50 in its homeodomain. Orthodenticle (Otd), also a K50 HD protein, is a Bcd transcriptional target. Bcd and Otd are expressed in overlapping patterns in Stage 5 syncytial blastoderm embryos. Here we present an analysis of *in vivo* binding activities of Bcd and Otd at two different developmental timepoints. ChIP-seq experiments show that Otd binds very few loci in stage 5 embryos (4% of the Bcd-bound peaks). However, the peaks shared by Bcd and Otd include the famous Bcd target gene *hunchback* (*hb*), and analyses of Hb in embryos lacking otd suggest that otd may function to help shape the Bcd activation gradient. At stage 6-8, there is a much stronger overlap between Bcd and Otd-bound peaks (59%). Most of the overlapping peaks are bound by Bcd early and Otd late, suggesting that Otd protein maintains gene expression of some early Bcd-activated targets through a relay mechanism. The peaks that are bound only by Otd late suggest that Bcd and Otd can bind different target enhancers despite recognizing the same TAATCC site. We present preliminary evidence that supports the hypothesis that Bcd- and Otd-specific cofactors are critical for the differential binding and activation of each protein's target genes.

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An Evolutionarily Conserved Polybasic Motif Mediates the Plasma Membrane Targeting of Lgl and Its Regulation by Hypoxia. Yang Hong, Wei Dong, Xuejing Zhang, Weijie Liu, Yi-jiun Chen, Juan Huang. Dept Cell Biol & Physiology, Univ Pittsburgh Med Sch, Pittsburgh, PA.

Lethal giant larvae (Lgl) was one of the first genetically identified tumor suppressors in *Drosophila* which was later also identified as a key regulator of cell polarity and asymmetric cell divisions. A prominent feature of Lgl is that plasma membrane (PM) localization appears to be critical for Lgl function in vivo and it is well characterized that phosphorylation of Lgl by polarity protein complex of aPKC (atypical PKC)/Par-6 inhibits Lgl membrane targeting and its activity during cell polarization and asymmetric cell division. However, molecular mechanisms mediating the PM targeting of Lgl remains unsolved. Here we report an unexpected discovery that hypoxia acutely and reversibly inhibits the PM targeting of Lgl in epithelial cells, through a post-translational mechanism that is modulated by the HIF pathway and intracellular ATP levels, but is independent of aPKC/Par-6 complex and cortical actin. Such observations led us to identify that in both *Drosophila* and mammalian cells the PM targeting of Lgl requires a conserved Arg /Lys-rich polybasic motif which also contains the conserved phosphorylation sites of aPKC. Our data show that the electrostatic binding between the positively charged polybasic motif and negatively charged phospholipids on the PM directly targets Lgl to PM. Such polybasic motif-mediated PM targeting provides an essential molecular mechanism for the regulation of Lgl functions either by hypoxia that diminishes the biosynthesis of phospholipids through reducing intracellular ATP levels, or by aPKC-mediated phosphorylations that neutralize the charges on the Lgl polybasic motif.*Drosophila* mutants with positive charges on the polybasic motif removed on Lgl show loss of function phenotypes similar to lgl^{null}, confirming the polybasic motif-mediated PM targeting mechanism is essential of Lgl function. Further, reducing Lgl expression levels in HEK293 cells increased cell viability under hypoxia, suggesting inhibition of Lgl PM targeting by hypoxia could promote tumorigenesis by repressing Lgl tumor suppressor.

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Huntingtin transports a novel class of vesicles on *Drosophila* larval axons. J. White, E. Anderson, K. Zimmerman, K.H. Zheng, R. Rouhani, T. Kelsang, S. Gunawardena. Biological Sciences, University at Buffalo, Buffalo, NY.

Huntingtin (HTT), the Huntington's disease (HD) protein, has been suggested to function during axonal transport. Previous work showed that loss of HTT function caused axonal transport defects. Biochemical and genetic experiments indicated that HTT can associate with both kinesin-1 and dynein motors either directly or via accessory proteins. However, the vesicle type or motor-cargo complex that contains HTT during axonal transport is unknown. Using *in vivo* motility analysis in Drosophila larval axons, we found that HTT influences the movement of particular Rab containing-vesicles. Strikingly, while reduction of HTT dramatically perturbed the bi-directional movement of Rab3, Rab4, and Rab19-containing vesicles, only the retrograde movement of Rab7-containing vesicles was perturbed. Using dual-color imaging, we then examined whether HTT is physically present on moving Rab-containing vesicles. Rab4 is present on most of the HTT containing vesicles, but not all Rab4 vesicles contained HTT. Rab3, Rab19, and Rab7 have been shown to identify with synaptic vesicles, recycling, and late endosomes, respectively. However, the identity of Rab4-containing vesicles is unknown. Dual-color imaging revealed that Rab4 is also present on synaptotagmin and synaptobrevin-containing vesicles indicating that Rab4 is likely to be on synaptic vesicles. Interestingly, Rab4 and Rab3 are not parts of the same complex while some Rab19 co-localized with Rab4. Collectively, our *in vivo* analysis provides compelling evidence for a HTT-Rab4 motor-complex during axonal transport and suggests that HTT may likely influence the motility of different Rab-containing vesicles. Moreover, expansion of polyQ repeats in the context of HTT dramatically perturbed the motility of Rab4-containing vesicles indicating that axonal transport defects induced by pathogenic polyQ on Rab-containing vesicles could be a major cause of the neuronal defects observed in HD pathogenesis These findings have important implications for our understanding of the complex transport machinery that must exist *in vivo* to move several different motor-cargo complexes on microtubules.

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Maturation of cytokinetic ring and abscission in Drosophila polarized epithelial cells. Roland Le Borgne, Emeline Daniel, Irina Kolotueva. Institut de Génétique et Développement de Rennes, CNRS UMR 6290, Rennes, France.

Epithelia are compact tissues composed of juxtaposed cells that function as mechanical and chemical barrier between the body and the environment. The barrier function relies first on adhesive contacts within adherens junctions (AJ) that serves as a mechanical barrier, and second on tight junctions (vertebrates)/septate junctions (SJ) (invertebrates) that function as a diffusion barrier. Throughout development and adult life, as epithelial cells divide to allow tissue growth and tissue repair, the barriers functions need to be preserved to ensure the maintenance of tissue integrity. Using *Drosophila* dorsal thorax epithelia as a model system, we are combining time-lapse confocal imaging and Electron Microscopy (EM) studies to understand how this barrier function is maintained and transmitted during epithelial cells cytokinesis. During cytokinesis the contractile actomyosin ring bisects the belt of AJ, the ring closing below the AJ. We report that the midbody matures within the SJ, and migrates towards the basal pole. Strikingly, we found that neighbouring cells remains in tight contact with the newly formed midbody and this contact persist as long as maturing midbody resides within SJ. We propose and currently test that this four-cell contact is important to maintain the integrity of the diffusion barrier. EM revealed that the midbody is maturing into an intercellular bridge: this transition is characterized by microtubules disappearance from the midbody observed both in light and electron microscopy approaches. Live microscopy and FRAP analyses also reveals that whereas myosin, septins and SJ markers persist throughout mibdoby to intercellular bridge maturation, actin is removed shortly after ring closure. EM data demonstrate that the intercellular bridge is eventually internalized into one of the divided cells through the complex remodelling of the membranes, suggesting abscission. We are currently investigating the final step of cytokinesis using several mutants including the ESCRT complex previously proposed to play a role in abscission in isolated cells. Our work is aimed to obtain a comprehensive view of epithelial cell cytokinesis.

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***Detachment versus cohesion: novel roles for PDZ-GEF and Rap1 during collective cell migration*. Ketki Sawant**[1,2], George Aranjuez[2,3], Jocelyn McDonald[1,2,3]. 1) 1Department of Biological, Geological and Environmental Sciences, Cleveland State University, OH; 2) Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic , OH; 3) Department of Genetics, School of Medicine, case Western Reserve University, OH.

Collective cell migration is a frequent mode of cell navigation in cancer, tissue repair and morphogenesis. During *Drosophila* oogenesis, 6-8 follicle cells are specified to form the border cell cluster. Border cells then detach from the follicular epithelium and migrate as a cohesive cluster from the anterior to posterior end of the egg chamber. However, the mechanisms that control cluster detachment and migration as cohesive group are still poorly characterized. Here we report that the small GTPase Rap1 along with its activator PDZ-GEF (PDZ domain-containing guanine nucleotide exchange factor) together have opposing functions during the detachment and cluster cohesion of migrating border cells. Loss of *PDZ-GEF* or *Rap1* each impaired cell adhesion between border cells and caused the cluster to partially splay apart; this in turn strongly disrupted the ability of mutant border cells to complete their migration. Live imaging of dominant-negative Rap1 border cells confirmed that mutant border cells no longer behaved as a cohesive cluster, and instead individual border cells became more protrusive before they stopped migrating. These data support the idea that Rap1 activity aids in adhesion, which is critical for border cells to couple together and communicate as a group during migration. Conversely, high PDZ-GEF or Rap1 activity each prevented detachment of border cells from the epithelium, presumably through abnormal maintenance of adhesion between border cells and follicle cells. We propose that Rap1 activity needs to be low specifically at border cell-follicle cell junctions when border cells detach from the epithelium, but high between border cells during migration to maintain cluster cohesion. We are currently testing this hypothesis using a newly developed *in vivo* Rap1-FRET activity assay. Thus, PDZ-GEF activates Rap1 to differentially promote cell adhesion and cohesion of the border cell cluster during collective cell migration.

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Linking epithelial apical-basal polarity to cell height determination via the microtubule minus end protector Patronin. Michiko Takeda, **Yu-Chiun Wang**. Laboratory for Epithelial Morphogenesis, RIKEN Center for Developmental Biology, Kobe, Hyogo, Japan.

Epithelial cells shapes are diverse. The distinct height-to-width ratios that manifest in the squamous, cuboidal and columnar epithelial cells are crucial for their physiological functions. The height dimension of epithelial cells, also known as the apical-basal axis, is invariably polarized. Although the molecular mechanisms underlying epithelial polarization have been extensively studied, whether and how it influences the cell height remains poorly understood. We use a process of epithelial folding during *Drosophila* gastrulation, dorsal fold formation, as a model system to investigate the interplay between modification of apical-basal polarity and alteration of cell height. Dorsal fold formation is initiated by downregulation the MARK family kinase Par-1, which leads to basal shifts of adherens junctions that ultimately cause the cells to shorten and the epithelial tissue to bend. Par-1 downregulation mediates junctional shifts by allowing its substrate, the Par-3 scaffolding protein Bazooka, to progressively colonize membrane surfaces basal to its previous sites of localization. Moreover, the microtubule minus end-oriented, apical translocation of Bazooka appears to also play a critical role. RNAi knockdown of Patronin, the CAMSAP family minus-end protector, causes destabilization of microtubules, resulting in widespread, premature basal junctional shifts, and formation of ectopic epithelial folds. Patronin localization transitions from a perinuclear region to the apical cortex as the polarity becomes established, where it likely stabilizes non-centrosomal microtubule minus ends to ensure the apical delivery of Bazooka. Furthermore, such apical localization is suggestive of an interaction with the apical membrane skeleton βHeavy-Spectrin that may directly control apical cell shape. Thess observations suggest an interactive network centered on the CAMSAP-dependent

microtubule dynamics that links epithelial apical-basal polarity and junction positioning to the shaping of apical cortex and the control of epithelial cell height.

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An essential morphogenetic role for Integrins in regulating tissue level tensile force by modulation of cell mobility. Emily Lostchuck[1], Stephanie J. Ellis[1], Katharine E. Goodwin[1], Teresa Zulueta-Coarasa[2], Daniela Gunne[1], Sabrina Wistorf[1], Rodrigo Fernandez-Gonzalez[2], James Feng[3], Guy Tanentzapf[1]. 1) Cell and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Cell and Systems Biology at the University of Toronto, Ontario, M5S 3G5, Canada; 3) Dept of Mathematics, University of British Columbia, Vancouver, British Columbia, Canada.

Integrin-mediated Cell-ECM adhesion is essential for tissue morphogenesis. Integrins facilitate morphogenetic processes by supporting cell migration, stabilizing cellular architecture and by regulating cell behavior. Here we describe an additional way that integrins contribute to morphogenesis: they ensure that the biomechanical parameters that exist within a tissue promote the desired morphogenetic outcomes. Our work focuses on Dorsal Closure (DC), an integrin-dependent morphogenetic process that occurs during fly embryogenesis. We have identified novel integrin-containing adhesive structures on the basal surface of the amnioserosa, a tissue that is essential for DC. As these structures share many features with focal adhesions we termed them Focal Adhesion-Like Structures (FALS). Using mutations that either increase or decrease integrin-based Cell-ECM adhesion, we show that integrins regulate the mobility of amnioserosa cells within the two-dimensional epithelial sheet. Furthermore FALS morphology directly correlates with strength of Cell-ECM adhesion. Using particle velocimetry, image analysis, and *in vivo* laser ablation experiments we find a direct correlation between cell mobility, FALS morphology and control of tension in the tissue. We show that mutations that alter cell mobility and tension within the amnioserosa result in defective DC. We are able to corroborate this data using a biomechanical mathematical model of DC. Overall, our data demonstrates that integrin-mediated cell-ECM adhesion determines cell mobility, and consequently, tension within a tissue and that this regulation has profound effects on tissue morphogenesis. Our results therefore uncover a fundamental and likely conserved mechanism for developmental regulation that links integrin-based adhesion, tissue biomechanics, and morphogenesis.

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Combining imaging and genetics to elucidate how integrin adhesion sites are built. Nicholas H. Brown[1,2], Natalia Bulgakova[1,2], Annabel G.M. Griffiths[1,2], Yoshiko Inoue[1,2], Maddy Parsons[3], Robert Stojnic[2]. 1) Gurdon Institute, Univ Cambridge, Cambridge, UK; 2) Dept of Physiology, Development and Neuroscience, Univ Cambridge, Cambridge, UK; 3) Randall Division of Cell and Molecular Biophysics, King's College London, London, UK.

Integrin-mediated adhesion to the extracellular matrix contributes to many aspects of of Drosophila development and homeostasis. At sites where integrins form strong stable adhesive junctions, the binding of integrins to extracellular ligands triggers the assembly of an intracellular complex containing more than 15 proteins. Using a combination of genetics and imaging we aim to discover how each of these proteins contributes to integrin function, how the structure is assembled, and how the stoichiometry of the components is controlled. Three lines of experimental analysis have revealed how interactions between the components are regulated and contribute to the construction of the adhesion complex.

Firstly, examining how the modest reduction of each component in a heterozygous mutant impacted on the levels of 9 other components, using quantitative confocal imaging, resulted in a complex data set that demonstrates the combinatorial nature of the recruitment pathways. We used computational modelling to identify which of all the possible networks of interaction can reproduce the data set most accurately. The best-fitting models involve distinct, parallel complexes downstream of integrins. Secondly, we examined how altering force affects the level of recruitment, with the unexpected result that reducing force in different ways, by eliminating innervation, "non-muscle" myosin II or muscle myosin, resulted in very different alterations to the stoichiometry of the complex. Thirdly, we have used FRET-FLIM to image specific interactions between components at the adhesion site. To our surprise, the known interactions between ILK, PINCH and parvin form slowly and progressively at the adhesion site, and require contractile force for their formation. Thus, the force-dependent pathways that mediate interactions between the components begin to reveal why integrin function requires such complex intracellular machinery.

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Malignant *Drosophila* tumors interrupt insulin signaling to induce cachexia-like wasting. Alejandra Figueroa-Clarevega, David Bilder. Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Although the importance of local interactions between a tumor and its microenvironment are well-known, how the tumor also interacts with more distant host tissues remains poorly understood. These long-range effects play a large role in morbidity and mortality, particularly in cachexia, a wasting condition that debilitates many cancer patients. Despite its prevalence in cancer pathophysiology, cachetogenic mechanisms have remained elusive; deciphering them could lead to new therapeutic inroads. Transplantation of the malignant $scrib^{1}/Ras^{V12}$ tumor model into an adult female induces a dramatic cachexia-like wasting of muscle, adipose and gonadal tissues. Despite the similarity between these phenotypes and those observed under starvation, tumor-bearing flies feed normally. Nonetheless, insulin activity is reduced in the affected peripheral tissues of tumor-bearing hosts. We have exploited the observation that malignant but not benign tumors can induce wasting to test malignant-specific secreted factors that drive the cachectogenic response. We identified the Insulin Growth Factor Binding Protein (IGFBP) homolog ImpL2, an antagonist of insulin signaling, as a tumor-secreted factor mediating the wasting response. ImpL2 secretion is sufficient to drive tissue loss and importantly, knocking down *ImpL2* specifically in the tumor ameliorates wasting phenotypes. We propose that tumor-secreted ImpL2 creates insulin

resistance in distant tissues and thus drives a systemic wasting response.

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Tumor progression by a genetic heterogeneity of cell clones with distinct oncogenic activities. M. Enomoto[1], D. Takemoto[1], T. Igaki[1,2]. 1) Laboratory of Genetics, Graduate School of Biostudies, Kyoto University, Japan; 2) PRESTO, Japan Science and Technology Agency (JST), Japan.

Tumor heterogeneity plays an important role in cancer progression, but its underlying mechanism is poorly understood. We have previously found that clones of cells activating oncoprotein Src in the eye disc cause non-autonomous overgrowth of surrounding tissue. This suggests a possible mechanism of tumor progression through an interaction of cell clones with distinct oncogenic activities. Here, we screened oncogenes that can promote epithelial tumor progression through cell-cell interactions with Src-activating clones in the eye disc and identified oncogenic Ras as a driver of non-autonomous tumor malignancy. Ras-activated cells cause neighboring Src-activated cells to induce metastatic behaviors such as invasion to the ventral nerve code. We found that Notch is upregulated in Src-activated clones, while the Notch ligand Delta is upregulated in Ras-activated clones, suggesting the possible role of Notch signaling in regulating this interclonal oncogenic cooperation. Our observations suggest that a genetic heterogeneity in Src and Ras activities within an epithelium can trigger tumor progression. The mechanism underlying this interclonal oncogenic cooperation will be discussed.

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Conserved Rab-11 regulation of intestinal inflammation as potential therapeutic targets in colon cancer. Yingchao Nie[1], Shiyan Yu[3], Alla Amcheslavsky[1], Qi Li[1], Zhong Jiang[2], Nan Gao[3], Tony Ip[1]. 1) Program in Molecular Medicine, UMASS Medical School, Worcester, MA; 2) Department of Pathology, UMASS Medical School, Worcester, MA; 3) Department of Biological Sciences, Rutgers University, Newark, NJ.

In searching for niches regulating the *Drosophila melanogaster* intestine stem cell homeostasis using RNAi, knockdown of the small GTPase Rab11 was found to cause strong intestine stem cell (ISC) proliferation and tissue hyperplasia. Enlarged stem cell nests and increased number of mitotic marker phosphor-histone 3 staining were observed. Analysis of differentially expressed genes using RNA-seq after Rab11 knockdown in enterocytes revealed increased production of inflammatory cytokines and growth factors such as Upd3, Spz, cv-2 and Vn, indicating the observed phenotype may due to the involvement of multiple pathways. We further examined the effect of decreasing Upd-3 expression on Rab11 RNAi induced phenotype, and found that loss of Upd-3 using a Upd-3-null mutant can rescue the Rab11-induced overproliferation by 50%, suggesting Upd-3 directly contribute to the hyperplasia phenotype. We hypothesize that Rab11 functions to maintain the intestinal homeostasis by keeping the ISC niches in an appropriate state, and loss of Rab11 causes inflammation-induced hyperplasia. Consistent with this idea, the increased inflammatory cytokines and proliferation were also observed in mouse*Rab11a* knockout intestine, indicating Rab11 regulatory role in the inflammation-induced hyperplasia is evolutionarily conserved. We therefore speculate that Rab11 expression may be down-regulated in human intestinal cancers. In supporting of our idea, we found stage III colon cancer patients have significantly lower level of Rab11 protein expression compared with stage I and II, indicating loss of Rab11 may play a role in cancer progression and metastasis.

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Using *Drosophila* to Develop Drug Cocktails to Treat Multiple Cancer Networks. T.K. Das, J. Esernio, R.L. Cagan. DRB, Mount Sinai School of Medicine, New York.

Cancer therapeutics seeks to identify potent drug cocktails as well as novel inhibitors with maximal therapeutic index. Cell-line based screening methods are not always predictive of optimal therapeutic index. They cannot effectively identify drugs that restrain cancer networks yet keep normal cell networks relatively unperturbed. Using a whole animal *Drosophila* model of *Multiple Endocrine Neoplasia Type II* (*MEN2*) we tested single drugs as well as combinations. Our matrix included 33 drugs either approved or in clinical trials for *MEN2* and other cancers. We identified a 2-drug combination that showed more potent suppression of *MEN2* cancer line viability than individual drugs targeting the driver oncogene *Ret*. Using modifier screens we identified additional pharmacologically druggable targets to improve therapeutic index in a 3-drug cocktail. To ascertain the effect of cocktails on cells we profiled the status of signaling networks in both transformed and normal fly tissues. The 3-drug cocktail activated fewer signaling pathways in both tissues compared to the single primary Ret inhibitors, and also improved overall viability, suggesting that the additional drugs kept primary-drug induced network feedback under control. This cocktail also suppressed *MEN2* cancer cell line growth in mouse xenograft studies. The ability to restrain drug-induced network-feedback in normal tissues indicated that components of this cocktail could be useful in other cancers. Growth suppression of other human lung, liver, breast, thyroid cancer lines treated with primary standard-of care drugs improved considerably in the presence of these additional drugs. Reduction of IC_{50}'s of standard of care drugs in these cancer lines ranged from 10-100 fold, suggesting ability to achieve potent growth suppression with much lower drug concentrations, a potentially useful quality of life benefit for patients. We have combined whole animal drug screening in *Drosophila* with normal vs transformed cell signaling activity profiling, to identify modular drug cocktails that work across a variety of human cancers. We show that balanced inhibition of entire cancer networks yield the most optimal long-lasting efficacy and also minimize normal cell toxicity.

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Mechanism of the metabolic shift induced by activating oncogenic pathways in *Drosophila* tumorigenesis. Cheng-Wei Wang[1], Arunima Purkayastha[1], Kevin T. Jones[1], Wei Liao[1], Utpal Banerjee[1,2,3,4,5]. 1) Department of Molecular, Cell and Developmental Biology; 2) Department of Biological Chemistry; 3) Molecular Biology Institute; 4) Broad Stem Cell Research Center; 5) Minor in Biomedical

Research, UCLA, Los Angeles, CA 90095.

The study of cancer cell metabolism holds the promise to unlock the energetic underpinnings of tumorigenesis. Because of the genetic complexity of tumors, it is imperative to establish simplified model systems in which the connections between oncogenic signals and metabolic components can be elucidated. We undertake genetic experiments in the *Drosophila* wing imaginal disc aimed at resolving how diverse oncogenes affect cell metabolism. We find that activation of oncogene PDGF- and VEGF-receptor (PVR) leads to the stabilization of sima, the *Drosophila* homolog of hypoxia-inducible factor-1 alpha (Hif-1α), ultimately resulting in a metabolic shift to aerobic glycolysis. RNA sequencing analysis reveals that sima controls this metabolic shift through elevating the transcription of several glycolytic genes. In addition to transcriptional up-regulation of glycolytic genes, the levels of Pyruvate Dehydrogenase Kinase (PDK) protein, which is a negative regulator of Pyruvate Dehydrogenase (PDH) in mitochondrial matrix, also increases upon PVR activation. Our experiments suggest that, the function of PDK is regulated through JNK signaling, which is activated upon PVR activation. The inactivation of PDH by PDK blocks the Krebs cycle and disrupts mitochondrial oxidative phosphorylation, resulting in high levels of reactive oxygen species (ROS) generated from mitochondria. Furthermore, we demonstrate that ROS acts as a feedback signal to further promote the metabolic shift. Our work probes the mechanism of oncogenic signal-mediated metabolic shift, and establishes *Drosophila* as a useful model system for understanding how oncogenic signals alter metabolic activities.

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Obesity-induced Cardiac Dysfunction in Starvation-Selected *Drosophila*. Christopher Hardy[1], Ryan Birse[2], Matthew Wolf[3], Allen Gibbs[1]. 1) School of Life Sciences, University of Nevada Las Vegas, 4505 S. Maryland Pkwy., Las Vegas, NV. 89154; 2) Development, Aging and Regeneration Program, Sanford Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA. 92037; 3) School of Medicine - Cardiology, Duke University, DUMC 103208, Durham, NC, 27710.

Drosophila has recently emerged as a model to study the pathophysiological effects of obesity on the heart. When fed high fat or sugar diets, *Drosophila* become obese, leading to heart defects including dilation, steatosis and myofibrillar disorganization. We have selected populations of *Drosophila* for starvation resistance for over 85 generations. In response, the starvation-selected (SS) lines have evolved physiologies that mimic obesity in mammals, including much higher lipids and lower metabolic rates than their unselected controls. We demonstrated a direct relationship between fat storage and heart dysfunction, as we rescued the SS hearts by making them lean through a period of starvation. These findings suggest that the genetic basis of heart disease in the SS lines is dependent on lipid homeostasis. We hypothesize the major source of dysfunction to be the physical interference of hypertrophic adipose tissue between the dorsal cuticle and the heart, which we observed upon dissection. We found no evidence of cardiac steatosis in the SS lines, nor any indication of myofibrillar disorganization. These data suggest that the mechanism of heart dysfunction in the SS lines is fundamentally different from previously reported models of obesity induced heart disease in *Drosophila*. Overall our findings provide a new model to study the physiology and genetics of obesity-induced heart disease.

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Muscle specific depletion of *twinstar* in *Drosophila* phenocopies Nemaline Myopathy. Mridula Balakrishnan[1,2], Shannon F. Yu[1], Mary K. Baylies[1]. 1) Developmental Biology, Sloan Kettering Institute, New York, NY; 2) Weill Cornell Graduate School of Medical Sciences, New York, NY.

Nemaline Myopathy (NM), a congenital myopathy, is characterized by the presence of rod-like structures, called nemaline bodies, in myofibers. NM patients display hypotonia and muscle weakness, particularly in the proximal, respiratory and facial muscles. No cure exists. Nemaline bodies consist of accumulations of Z-disc proteins of sarcomeres, the contractile units of a myofiber. Cofilin-2, a member of Actin Depolymerizing Factor (ADF) family of proteins, is mutated in NM patients. *twinstar(tsr),* the *Drosophila* homologue of *Cofilin-2,* is highly expressed in *Drosophila* muscle throughout development. Here we show that *tsr* depletion affects late stages of muscle development; specifically reduction of *tsr* leads to sarcomeric abnormalities and formation of nemaline bodies in larval muscle. The nemaline bodies compromise both muscle structure and function, assayed through Confocal/ Electron Microscopy and crawling assays respectively. Together these deficits result in organismal lethality. Furthermore, expression of full-length Tsr protein in *tsr*-depleted muscle restores muscle function and structure. When a human disease variant of *tsr* is expressed in larval muscles, we find that the disease variant correctly localizes, but the conserved sarcomere structure is still affected, indicating the importance of Tsr in maintaining the integrity of the sarcomere. The range of phenotypes observed as well as the similarity in the pathophysiology with that of the human disease indicate that we have developed a *Drosophila* model of Nemaline myopathy which will allow further mechanistic studies of NM and identification of therapeutics for this disease.

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Transcriptional activation by a low-complexity domain is a conserved feature of Zelda and orthologous proteins. Danielle Hamm, Eliana Bondra, Melissa Harrison. Dept. of Biomolecular Chemistry, University of Wisconsin, Madison, WI.

In all animals, maternal gene products regulate the initial events of embryogenesis. Hours after fertilization development switches from maternal to zygotic control at a time point known as the maternal-to-zygotic transition (MZT). The transcription factor Zelda (ZLD) plays a key role in the global transcriptional activation of the zygotic genome in *Drosophila melanogaster*. ZLD is a large protein with six C2H2 zinc fingers but no additional identifiable domains or predicted enzymatic activity. To better understand the mechanism of ZLD-mediated transcriptional activation, we defined the functional domains of ZLD required for both DNA binding and transcriptional activation. Splice isoforms present in larvae lack three of four C-terminal zinc-fingers required for DNA binding and dominantly suppressed transcriptional activation by the full-length isoform. These truncated isoforms contain the transcriptional activation domain

of ZLD, which we mapped to a central region characterized by low complexity. Despite little primary sequence conservation of this activation domain within insects, ZLD orthologs drive transcription in *D. melanogaster* cells. Based on these data, we propose that ZLD functions through conserved interactions with a protein cofactor(s) to mediate the initial activation of the zygotic genome and that expression of truncated isoforms can modulate this activity. .

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Discovery of Novel Enhancers Using Natural Variation. Ashley Jermusyk, Sarah Gharavi, Gregory Reeves. Chemical and Biomedical Engineering, North Carolina State University, Raleigh, NC.

A complex system of gene regulatory circuits controls the signaling processes involved in tissue patterning. These circuits act to buffer the developing pattern against noise, thereby minimizing mistakes in gene expression and preventing patterning defects. One of these gene regulatory networks patterns the anterior-posterior axis of the developing *Drosphila* embryo. Much work has been done to find the binding sites and enhancer regions that properly position gene expression domains within this system. This work has generally been performed using brute force methods; however we propose a new method to discover these enhancers and important gene regulatory regions. Our method explores differences in natural gene expression due to subtle genomic differences. We used a selection of wild-caught lines and quantified differences in positioning of gene expression boundaries of *Krüppel* and Even-skipped domains and in the transcriptome. The differences in the positions of *Krüppel* and Even-skipped were then correlated to genomic differences to find novel regulatory regions. We were then able to test and validate these enhancer regions using enhancer traps. By determining the transcriptome of the early embryo we were able to correlate differences in gene expression levels to genomic differences within enhancer regions. In this way novel genes and DNA regulatory elements can be identified which have functional interactions within the network that patterns the anterior-posterior axis of the *Drosophila* embryo.

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Shadow enhancers enable Hunchback bifunctionality in the *Drosophila* embryo. M. Staller, B. Vincent, M. Bragdon, J. Estrada, Z. Wunderlich, **A. DePace**. Systems Biol, Harvard Med Sch, Boston, MA.

Hunchback (Hb) is a bifunctional transcription factor that activates and represses distinct enhancers. We investigated the hypothesis that Hb can activate and repress the *even-skipped (eve)* stripe 3+7 enhancer in *Drosophila* blastoderm embryos, as has been proposed by previous computational studies. We measured and modeled *eve* expression at cellular resolution under multiple genetic perturbations and found that the *eve3+7* enhancer could not explain endogenous *eve* stripe 7 behavior. Instead, we found that *eve* stripe 7 is controlled by two enhancers: the canonical *eve3+7* and a sequence encompassing the minimal *eve* stripe 2 enhancer (*eve2+7)*. Hb bifunctionally regulates *eve* stripe 7, but it executes these two activities on different pieces of regulatory DNA—it activates the*eve2+7* enhancer and represses the *eve3+7* enhancer. These two "shadow enhancers" therefore use different regulatory logic to create the same pattern and create a complex transcriptional circuit. I will discuss additional examples of shadow enhancers that use different regulatory logic and the possible functional roles of these types of transcriptional circuits. .

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Changes in a P-MAD binding site underlie species diversity of *wishful thinking* patterning. Rob Marmion[1], Milica Jevtic[2], George Pyrowolakis[2], Nir Yakoby[1]. 1) Biology Department and Center for Computational and Integrative Biology, Rutgers University, Camden, NJ; 2) Institute for Biology I, Albert-Ludwigs University of Freiburg, Freiburg, Germany.

The *Drosophila* eggshell is an established model to study cell signaling, tissue patterning, and morphogenesis. The bone morphogenetic protein (BMP) signaling pathway is a crucial regulator of tissue growth during multiple stages of *Drosophila* development. We found the type II receptor, *wishful thinking* (*wit*), to be dynamically and non-uniformly expressed in the follicle cells, which are a mono-layer of epithelial cells engulfing the developing oocyte. Wit is necessary for BMP signal transduction and is regulated transcriptionally by BMP signaling within the follicle cells. We studied gene regulation by BMP via studying the *cis*-regulatory module (CRM) responsible for *wit* expression. Here we describe a binding site for P-MAD, the transcription factor of the BMP pathway, which differs from the literature description. We utilized *D. virilis*, a species separated by 45 million years of evolution from *D. melanogaster*, to study the evolution of *wit* regulation. Interestingly, *wit* patterning differs both in width and dynamics between the species. We recapitulated this pattern in *D. melanogaster* by expression driven by the homologous CRM. We suggest that spacing between MAD & MED binding sites underlies the patterning differences.

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A genetic screen for new Polycomb group genes. James Kennison, Monica Cooper. Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Genetic studies first identified the Polycomb group genes by their defects in transcriptional silencing of the homeotic genes. To identify new Polycomb group genes, we have developed a transgene assay using pairing-sensitive silencing of the mini-white reporter gene caused by a Polycomb Group Response Element (PRE) from the Sex combs reduced homeotic gene. Recessive mutations that disrupt silencing are recovered in mitotic clones in heterozygous flies. We have screened about 98% of the genome and isolated mutations in most of the known Polycomb group genes. We have also isolated mutations in several new genes required for silencing. Using a combination of meiotic recombination mapping and whole genome sequencing, we are determining the transcription units corresponding to these new silencing genes.

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Super-resolution imaging of chromatin nanostructure reveals tight coupling of epigenetic state and 3D genome organization. Alistair Boettiger[1], Bogdan Bintu[2], Jeff Moffitt[1], Brian Beliveau[4], Chao-ting Wu[4], Xiaowei Zhuang[1,2,3]. 1) Chemistry and Chemical Biology, Harvard University, Cambridge, MA; 2) Department of Physics, Harvard University, Cambridge, MA; 3) Howard Hughes Medical Institute, Harvard University, Cambridge, MA; 4) Department of Genetics, Harvard Medical School, Boston, MA.

Extensive analyses of the genomes of multicellular organisms have shown that many of the regulatory features of genes are organized at the scale of kilobases to megabases. The structure of genomic chromatin at this scale however is poorly understood. Here, we will present a method to visualize structural organization of regulatory chromatin domains using a high density DNA painting approach coupled to super-resolution STORM imaging. We observe a rich diversity of spatial conformations across the nearly 100 genomic loci we have imaged and quantified. Interestingly, we find several aspects of this structural diversity which correlate strongly with nature of the local epigenetic marks. For example, both the median volume occupied by a chromatin region and the folding organization of chromatin within that volume differ substantially and predictably depending on the epigenetic chromatin state. Of these, Polycomb bound regions exhibit an especially surprising spatial organization, in which larger domains are more tightly compacted than smaller domains and chromatin folding consists of irregular, long range loops rather than accordion like compaction. This organization also leads to spatial exclusion of neighboring chromatin from the volume bound by Polycomb. To better understand the molecular mechanisms responsible for the different patterns of folding, scaling, and segregation we see, we use computational models of the chromatin polymer. Together, these results demonstrate a high degree of structural organization at some of the most developmentally important gene clusters, which is likely relevant in determining their expression behavior. The method we introduce provides a new avenue to probe structure-function relationships within the genome and better understand gene expression. .

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The RNA binding protein Arrest (Aret) regulates myofibril maturation in *Drosophila* flight muscle. M. Spletter[1], C. Barz[1], A. Yeroslaviz[1], C. Schönbauer[1], D. Gerlach[3], I. Ferreira[1], M. Sarov[4], A. Stark[2], B. Habermann[1], F. Schnorrer[1]. 1) Max Planck Institute for Biochemistry, Martinsried, Germany; 2) Research Institute of Molecular Pathology, Vienna, Austria; 3) Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria; 4) Max-Planck-Institute of Cell Biology and Genetics, Dresden, Germany.

In *Drosophila*, fibrillar flight muscles (IFMs) enable flight, while tubular muscles mediate other body movements. *spalt major* (*salm*) was recently identified as a key regulator of the fibrillar fate, controlling expression of IFM-specific genes. Here, we systematically investigate differential isoform expression between fibrillar and tubular muscle using RNA-seq and isoform-specific reporters, showing that *salm* regulates a large set of sarcomeric proteins and thus determines fibrillar muscle physiology. Mechanistically, we identify the RNA binding protein Arrest (aret, Bruno), as downstream of *salm*. Aret shuttles between cytoplasm and nuclei, and is essential for myofibril maturation and sarcomere growth of IFMs. Molecularly, Aret regulates transcription and alternative splicing of a large subset of fibrillar muscle-specific isoforms, many of which are incorporated into the growing sarcomere during myofibril maturation. Aret regulated targets such as *wupA* (TnI), *up* (TnT) and *mhc* are implicated in muscle hyper-contraction phenotypes. The fiber degeneration phenotype upon *aret* knock-down can be rescued by removing *mhc* function from IFMs, confirming that loss of *aret* indeed induces hyper-contraction. We additionally identify an IFM-specific isoform of the titin-like gene*Stretchin* (*Strn-Mlck*) as an *aret* target. Interestingly, loss of this Strn-Mlck isoform also results in non-functional IFMs that degenerate in adults, suggesting that Strn-Mlck is a key target of *aret* necessary for the generation of functional IFMs. Together, our data demonstrate that the RNA binding protein Aret is a fundamental regulator of muscle-specific splicing and expression of core sarcomeric components, many of which are present in various isoforms and required in a particular combination to determine the mechanical and physiological properties of muscle subtypes. As Aret and its sarcomeric targets are evolutionarily conserved, similar principles may regulate mammalian muscle morphogenesis..

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Ultraconserved core elements are an essential feature of insect enhancers. Thomas Brody, Ward Odenwald. Neural Cell-Fate Determinants Section, NINDS/NIH, Bethesda, MD.

Existence of ultra-conserved sequence elements in vertebrate enhancers suggests that transcription factor regulatory interactions are shared across phylogenetically diverse species. To date evidence for similarly conserved elements in insect species has been elusive. Here, we have taken advantage of the availability of the assembled genomic sequence of the Mediterranean fruit fly *Ceratitis capitata*, the domestic housefly *Musca domestica*, and *Drosophila* to explore the integrity of regulatory sequences across the ~100 million years distance of these species. Using various alignment techniques we show that the many of the conserved sequence blocks (CSBs) that constitute *Drosophila* enhancers, identified using the multispecies alignment algorithm EvoPrinter, are often nearly completely conserved in both*Ceratitis* and *Musca*. Analysis of the linear positioning of these CSBs with respect to the associated structural genes is conserved as well. The results suggest that CSBs represent the point of interaction of multiple trans-regulators whose functions and interactions are conserved across divergent genera. We similarly sought sequences in bee and mosquito sequences that were related to those shared by*Drosophila, Ceratitis* and *Musca*, however none were found. Nevertheless, clusters of CSBs were found shared in the following pairwise comparisons of mosquito and bee species; 1) the mosquitoes*Culex pipiens* and *Aedes aegypti*, 2) the mosquitoes *Anopheles gambiae* and *A. sinensis,* and 3) the bumble bee *Bombus impatiens* and honey bee *Apis mellifera*. In addition, alignment of *Apis* with the European bee *Megachile rotundata* reveals ultraconserved sequences embedded in *Apis* conserved sequence clusters. These studies will allow for a greater understanding and comparative analysis of gene regulation in these species.

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Wash interacts with Lamin and affects global nuclear organization. J. Verboon, H. Rincon-Arano, B. Sugumar, T. Werwie, D. Scalzo, V. Nandakumar, J. Delrow, M. Groudine, **S. Parkhurst**. Div Basic Sci, Fred Hutchinson Cancer Res Ctr, Seattle, WA.

The cytoplasmic functions of Wiskott-Aldrich Syndrome family (WAS) proteins are well established and include roles in cytoskeleton reorganization and membrane-cytoskeletal interactions important for membrane/vesicle trafficking, morphogenesis, immune response and signal transduction. WAS proteins act as effectors of Rho family GTPases and polymerize branched actin through the Arp2/3 complex. We identified Wash as a new member of the WAS family with essential cytoplasmic roles in early development. Studies in mammalian cells and *Dictyostelium* suggest that WASH functions primarily in a multiprotein complex that regulates endosome shape and trafficking in an Arp2/3-dependent manner. However, roles for classically cytoplasmic proteins in the nucleus are beginning to emerge, in particular as participants in the regulation of gene expression. We find that fly Wash is present in the nucleus where it plays a key role in global nuclear organization. *wash* mutant and knockdown nuclei disrupt sub-nuclear structures/organelles and exhibit the abnormal wrinkled morphology observed in diverse laminopathies. We find that nuclear Wash interacts with B-type Lamin (Lamin Dm0), and like Lamin, Wash associates with constitutive heterochromatin. Wash knockdown increases chromatin accessibility of repressive compartments and results in a global redistribution of repressive histone modifications. Thus, our results reveal a novel role for Wash in modulating nucleus morphology and in the organization of both chromatin and non-chromatin nuclear sub-structures.

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Compliance sensing by actomyosin self-organization determines the direction of tensile force during morphogenesis. Soline Chanet[1], Callie Miller[2], Bard Ermentrout[3], Eeshit Vaishnav[1], Lance Davidson[4], Adam Martin[1]. 1) Department of Biology, MIT, Cambridge, MA; 2) Bioengineering, University of Pittsburgh, Pittsburgh PA; 3) Mathematics, University of Pittsburgh, Pittsburgh PA; 4) Bioengineering, Developmental Biology, and Computational and Systems Biology, University of Pittsburgh, Pittsburgh PA.

Generating the proper shape of organs and organisms requires that cells generate forces with proper directionality to sculpt specific forms. Tensile forces that result from the contraction of apical actin filament (F-actin) meshworks by the motor Myosin II are critical to bend and fold epithelial tissues. In *Drosophila* the formation of a long, narrow epithelial fold or ventral furrow is associated with apical constriction of a strip of ventral cells and with anisotropic epithelial tension oriented along the length of the furrow. How individual cells, which are 100's of times smaller than the size of the tissue, are instructed to generate actomyosin-based tension in a specific direction that will elicit proper tissue form is unknown. We demonstrate that apical actomyosin meshworks in the *Drosophila* embryo respond to mechanical cues in the tissue; this allows force generation by individual cells to be determined by physical properties of the embryo, such as global shape. We propose a model explaining how actomyosin meshworks can direct tension along the path of most resistance to cell deformation, allowing cells to sense and respond to mechanical cues. Importantly, cells of a different tissue (posterior midgut) that utilize the same upstream biochemical signaling pathway to induce apical constriction, exhibit a different configuration of the actomyosin cytoskeleton and exert tension with different directionality, resulting in a cup-like indendation rather than a furrow. This demonstrates the versatility of a singular biochemical signal transduction pathway and force-generating apparatus, which can generate different force patterns and forms based on different mechanical context. Our work demonstrates how systems-level physical properties that result from embryo/organ shape and gene expression pattern can orient cellular force generation via a mechanical feedback loop that involves actomyosin meshwork self-organization.

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Endocytosis drives junctional and cytoskeletal protein redistribution to promote rapid embryonic wound repair. Miranda V. Hunter[1], Rodrigo Fernandez-Gonzalez[1,2,3]. 1) Cell and Systems Biology, University of Toronto, Toronto, Canada; 2) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada; 3) Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, Canada.

Epithelial tissues have an outstanding ability to repair wounds, which is particularly marked in embryos. Rapid embryonic wound repair is driven by a contractile actomyosin cable, which assembles around the periphery of the wound and contracts, acting as a "purse string" that drives wound closure. This process is accompanied by the removal of junctional proteins such as E-cadherin from the interfaces between wounded and adjacent cells, and by simultaneous clustering of junctional proteins at discrete points at the wound margin. However, the significance and mechanisms of the junctional redistribution that accompanies embryonic wound closure are unclear. We used *in vivo* time-lapse microscopy and quantitative image analysis to examine the role of junctional rearrangements during epidermal wound repair. We found that clathrin and dynamin, important components of the endocytic pathway, accumulated at the wound margin, suggesting a role for endocytosis in wound repair. Notably, when we blocked endocytosis using pharmacological and genetic approaches, we found that wound closure was significantly delayed. The defect in wound closure was accompanied by both impaired depletion of E-cadherin from the wound margin, as well as defective assembly of the actomyosin purse string. These data suggest that endocytosis mediates redistribution of junctional proteins at the wound margin, which is necessary for assembly of the purse string and efficient wound closure. Consistent with this, we quantified defective actin dynamics around the wound and a delay in wound closure when E-cadherin was overexpressed in the embryo, further signifying that E-cadherin levels at the wound margin must be tightly regulated for rapid wound repair. Our results suggest a novel relationship between cytoskeletal proteins, junctional components, and endocytosis, all of which cooperate to drive efficient wound closure.

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Dunk stabilizes the tensile actomyosin network at the leading edge of the cleavage furrows during *Drosophila* cellularization. Bing He[1], Adam Martin[2], Eric Wieschaus[1,3]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; 3) HHMI, Princeton University, Princeton, NJ.

In *Drosophila*, the formation of the cellular blastoderm is achieved by cellularization, a special form of cytokinesis during which plasma membrane invaginates from the surface of the embryo and partitions the syncytial nuclei into individual cells. During cellularization, a dynamic actomyosin network formed at the basal leading edge of the cleavage furrows and subsequently reorganizes into individual contractile rings that pinch off the cell basally. We find that the basal myosin network is established through a cortical flow of myosin that leads to myosin accumulation at the invagination front as the furrows invaginate. Using laser ablation, we demonstrate that basal myosin is already under tension while it organizes into an interconnected network. We identified a novel gene *dunk*, mutation in which leads to a rapid myosin loss shortly after its recruitment to the invagination front, causing myosin to deplete from the edges and accumulate at the vertices. Although a subsequent, Slam-dependent direct recruitment of myosin to the invagination front replenishes basal myosin, the initial myosin loss results in abnormal basal morphology, a delay in myosin ring formation, and uncoordinated furrow invagination. The expression of *dunk* is restricted at the blastoderm stage, and its protein product co-localizes with myosin at the invagination front. The localization pattern and the mutant phenotype suggest that Dunk is directly involved in stabilizing the actomyosin network at the invagination front during early cellularization. Computer simulation demonstrates that vertex-accumulation of myosin in *dunk* mutant embryos can be recapitulated by altering the rates of association and dissociation of myosin to and from the cortex, coupled with a positive feedback mechanism that reinforces the cortical association of myosin upon tension increase. We propose that Dunk promotes structural integrity of the basal actomyosin network by preventing myosin loss from the cortex, therefore help to maintain basal contractile symmetry at the invagination front.

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A Balance between Arf and Rho1 G Protein Pathways Patterns the Early *Drosophila* Embryo. Donghoon Lee, Tony Harris. Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada.

Cell division often generates cell types which pattern and shape the body of multicellular organisms. In the early *Drosophila* embryo, primordial germline cells (pole cells) form at the posterior pole. However, our understanding of mechanisms that control such biased cytokinesis is limited. Previously, we documented that the *Drosophila* cytohesin Arf-GEF Steppke antagonizes Rho1 pathways that otherwise promote membrane cytoskeleton activity. Here, we report that a locally-regulated balance between Arf and Rho1 G protein activities contributes to the formation of pole cells. Specifically, loss of Steppke results in a striking phenotype, in which cells form at both the anterior and the posterior poles of the embryo before overall cellularization. Although the anterior cells lack the pole plasm marker Vasa, they bud and form at the same time as pole cells and then asynchronously divide, as pole cell do. By performing a series of rescue and genetic interaction experiments, we determined that Steppke normally activates Arf G proteins and antagonizes Rho1 actomyosin pathways to inhibit the anterior cell formation. To test if Steppke antagonizes Rho1 during pole cell formation, we perturbed both pathways and assessed the effects on pole cell numbers. Our preliminary data show that Rho1 heterozygotes have reduced pole cell numbers, and that *steppke* knockdown can revert this effect, indicating that Steppke antagonizes Rho1 activity during normal pole cell formation. To test if elevating Steppke levels can inhibit pole cell formation, we overexpressed GFP-Steppke and observed a dosage-dependent reduction in pole cell numbers. This effect depended on its Arf-GEF function and was enhanced by perturbing a pole plasm activator of the Rho1 pathway, Germcell-less. This work suggests that tuning of the balance between Arf and Rho1 G protein activities contributes to pole cell formation. Thus, the early *Drosophila* embryo appears to be patterned by posteriorly-biased cytokinesis dictated by antagonistic endocytic and cytoskeletal activities. Recent reports of Arf G protein regulation of asymmetric cell division in *C. elegans* suggest that this mechanism may be common across metazoans.

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Cellular Mechanisms of Heart Morphogenesis and Lumen Formation in *Drosophila*. Georg Vogler[1], Jiandong Liu[3], Timothy W Iafe[4], Ede Migh[2], József Mihály[2], Rolf Bodmer[1]. 1) Development and Aging, Sanford Burnham Medical Research Institute, La Jolla, CA; 2) Biological Research Centre, Hungarian Academy of Sciences, Institute of Genetics, H-6726 Szeged, Hungary; 3) Dept. of Pathology and Laboratory Medicine, and McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; 4) New York University School of Medicine, New York, NY 10016.

The *Drosophila* embryonic heart is a key model system for understanding heart specification. Our previous studies indicate that heart morphogenesis requires Slit/Robo signaling, a function conserved in vertebrates. The mechanisms by which these and other signals control heart formation are still unknown. Due to its role in membrane dynamics, we investigated the role of the small GTPase Cdc42 during *Drosophila* heart development and found it to be required for cardiac cell alignment and heart tube formation. Mutant or constitutively active *Cdc42* in the developing heart causes improper cardioblast alignment and formation of multiple lumina, resp., suggesting that Cdc42 is required during discrete steps of cardiogenesis. Cell polarity and filopodia dynamics are unaffected by loss of Cdc42, therefore Cdc42 might have a different role during heart morphogenesis. To understand the regulation of Cdc42 and to identify new genetic interactors, we performed a genetic screen for modifiers of*Cdc42*. We identified the non-muscle myosin-II *zipper* to strongly interact with *Cdc42*. Zipper exhibits a dynamic localization pattern during cardiogenesis, which depends on Cdc42 function, but interestingly is independent of Slit/Robo. Activation of the formin-like proteins Dishevelled Associated Activator of Morphogenesis (dDAAM) and Diaphanous (Dia) produced defects similar to activated Cdc42, indicating that control of cell shape changes is a key

regulatory step during heart morphogenesis. *Cdc42* also shows strong genetic interaction with *dDAAM*, suggesting that cardiac morphogenesis is controlled by a novel pathway involving Cdc42, *dDAAM* / *Dia* and *Zip* acting together during cardiac cell shape changes and to orchestrate heart lumen formation.

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Myosin light chain phosphatase regulates actomyosin contractility in the *Drosophila* follicular epithelium. M.D. Martin-Bermudo[1], I. Grosheva[1], D. Gómez-Mígues[2]. 1) Dept Developmental Biol, CSIC, Seville, Spain; 2) 2Departamento de Física de la Materia Condensada, Univ. Autonoma de Madrid, Madrid, Spain.

Coordinated actomyosin driven cell contraction underlies organ shape generation during development. Positioning of the actomyosin contractile machinery within a cell determines the outcome of the contractile activity and as a result, tissue shape. The development of the follicular epithelium (FE) of the *Drosophila* ovary provides an excellent model system for understanding the genetic control of basal actomyosin contractility during organogenesis. Previous work has shown that FCs undergo periodic basal actomyosin contractions around stage 9, which are required for proper egg elongation. However, the mechanisms regulating these contractile structures are currently unknown. Here, we show that the negative contractility regulator Myosin Light Chain Phosphatase (MLCP) is a key regulator of basal contractility in FCs. First, we show that at stage 9, concomitant with the formation of basal contractile actomyosin arrays and with the onset of basal contraction, MLCP changes its localization from being apical and basal to just apical. In addition, we find that removal of MLCP from FCs results in premature formation of basal acto-myosin structures and periodic pulsations. These results strongly suggest that MLCP regulates the onset of basal actomysoin contractility in FCs. Our results also show that actomyosin pulsations become stochastic in MLCP knock-out FCs, suggesting that MLCP is also required for maintenance of the oscillatory behavior of FCs. Finally, we have performed a mathematical modeling of basal myosin dynamics in wild type and mutant conditions, which not only reproduced *in silico*experimentally observed cell behavior, but also reveal some insights into the mechanosensitive feedback circuit operating during basal cell pulsation. We are currently testing in living cells prediction made by the model.

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Coordinated cell area contractions drive the formation of new cell contacts in germband extension. Deqing Kong[1], Lars Reichl[2], Yujun Zhang[1], Fred Wolf[2], Jörg Groβhans[1]. 1) Institute for Developmental Biochemistry, Medical School, University of Göttingen, Justus-von-Liebig Weg 11, 37077 Göttingen, Germany; 2) Max Planck Institute for Dynamics and Self-Organization, Am Faβberg, 37077 Göttingen, Germany.

Epithelia cell rearrangement is important for many processes in morphogenesis. During germband extension in early gastrulation of Drosophila embryos, exchange of neighbors is achieved by junction remodelling that follows a topological T1 process. After constriction of dorsal-ventral junctions and fusion of two 3x vertices into a 4x vertex, a new junction forms in perpendicular, anterior-posterior orientation. This resolves the 4x vertex into two new 3x vertices. In contrast to the good understanding of the initial junction constriction, little is known about the formation of the new junction. We found that new junctions form non-autonomously depending on the coordination of surface area fluctuations of the immediate neighbours. Coordination of area fluctuations and formation of new junctions require the ER glucosyl transferase Xit and E-Cadherin.

We established a high throughput imaging pipeline, by which we recorded, segmented and analyzed more than 1000 neighbor exchanges in wild type and mutant embryos. We investigated correlations between surface areas, junction lengths, myosin-II and E-cadherin levels in cells neighboring rearrangement. We found that area changes anti-correlate between old and new neighbors. This anti-correlation is strongest during resolution of the 4x vertex and is reduced in embryos mutant for *xit* or RNAi-depleted for E-cadherin. As both types of mutant embryos are also impaired in the formation of new junction, coordination of areas fluctuations may be the driving force for junction extension. Xit encodes a conserved glucosyl transferase required for N-glycosylation in the endoplasmic reticulum. In *xit* embryos, N-glycosylation and expression levels of E-cadherin are affected. We propose that E-cadherin mediates coordination of cell area changes by setting up a mechano-transduction pathway between neighboring cells. .

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Neuroblasts build their own adhesive daughter cell niche through activation of PI3-Kinase signaling. Sarah Siegrist, Susan Doyle, Matthew Pahl. Biology Department, University of Virginia, Charlottesville, VA.

Neuroblasts are the *Drosophila* neural stems and divide asymmetrically throughout development to produce all neurons and glia present in the adult brain. We showed previously that a subset of brain neuroblasts, the mushroom body neuroblasts (MB NBs) which generate neurons important for memory and learning, are eliminated by apoptosis during late pupal stages. We show here that some apoptotic inhibited MB NBs relocate from the dorsal surface of the brain to the pars intercerebralis (PI), an anterior medial brain region rich in neurosecretory neurons, including those that secrete some of the drosophila insulin like peptides. NBs located within the PI region have increased rates of cell proliferation and persist longer than MB NBs remaining positioned on the dorsal surface, raising the possibility that the PI region serves as a "latent" niche providing trophic stem cell support. We show that levels of PI3K activation in MB NBs regulate re-positioning, but in the opposite manner as we initially anticipated. High levels of PI3K are required to keep MB NBs properly positioned on the dorsal surface in their native microenvironments, while low levels of PI3K activity drive MB NBs out. Furthermore, we show that PI3K activity is localized to the newly generated cell contact site between NB and daughter after mitosis is complete where it likely functions in assembly of Cadherin containing cell adhesion complexes. We propose that PI3K activation in NBs is required not only for sustained NB proliferation during development but also to keep NBs properly anchored within their native niches through adhesion to daughter cells and that this may be a general mechanism for stem cell positioning. .

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The conserved Misshapen-Warts-Yorkie pathway acts in the epithelial niche to regulate intestinal stem cell division. Qi Li[1], Shuangxi Li[2], Sebastian Mana-Capelli[3], Rachel J Roth Flach[1], Laura V Danai[1], Alla Amcheslavsky[1], Yingchao Nie[1], Satoshi Kaneko[1], Xiaohao Yao[1], Xiaochu Chen[1], Jennifer L Cotton[4], Junhao Mao[4], Dannel McCollum[3], Jin Jiang[2], Michael P Czech[1], Lan Xu[1], Tony Ip[1]. 1) Molecular Medicine, UMass medical school, Worcester, MA; 2) Department of Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX; 3) Department of Biochemistry and Molecular Pharmacology, UMass medical school, Worcester, MA; 4) Department of Cancer Biology, UMass medical school, Worcester, MA.

Somatic stem cells produce the various cell types that maintain the tissue integrity and function. Equally important is the stem cells have the ability to optimize their proliferation rate depending on the environment change. Similar to the mammalian intestine, the Drosophila adult midgut has resident stem cells that support growth and regeneration during normal turn over or after injury. In this process, the epithelial niche cells have essential roles in regulating the response of stem cells to the environment but the mechanism is not well understood. Here we show that the conserved germinal center protein kinase Misshapen acts in enteroblasts to restrict intestinal stem cell (ISC) division. Misshapen, a distant relative to the prototypic Warts activating kinase Hippo, interacts with and activates Warts to negatively regulate the activity of the transcriptional co-activator Yorkie, which in turn regulates the expression of Upd3. The mammalian Misshapen homolog MAP4K4 similarly interacts with LATS (Warts homolog) and promotes the inhibition of YAP (Yorkie homolog). Further, changing cytoskeleton dynamics can modulate this conserved pathway. Together, our work reveals that the novel Misshapen-Warts-Yorkie pathway acts in enteroblasts to control niche signaling to ISCs. These findings also provide a model in which to study requirements for Misshapen/MAP4K4-related kinases in Hippo/MST1/2-independent regulation of Warts/LATS and Yorkie/YAP. .

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Identification of Novel Factors Required for Triggering the Drosophila Intestinal Stem Cell Response to Tissue Damage. Julieta A. Maldera, Marwa Elrefaey, Christine Gläßer, Bruce A. Edgar. DKFZ-ZMBH Alliance, Heidelberg, Germany.

Maintenance of epithelial homeostasis relies on a tightly coordinated process that involves the balance between removing damaged cells and producing new cells from resident stem cells. Epithelial cells of the digestive tract of most animals are short-lived and are constantly replenished by the progeny of intestinal stem cells (ISCs). As in humans, the integrity of the Drosophila gut epithelium is preserved by ISCs, by means of their ability to self-renew and produce differentiated cells. Interestingly, ISCs can change their division rate according to tissue needs, such as during injury. The stressed gut produces Unpaired cytokines (Upd, Upd2 and Upd3) as well as Epidermal Growth Factor Receptor (EGFR) ligands (Krn, Spi and Vn) that promote compensatory ISC division and differentiation, leading to tissue regeneration. However, how the intestinal epithelium detects damage and translates this signal into a regenerative response remains poorly understood. We aimed to extend this research by identifying genes activated in response to cell loss that act as signaling molecules to translate the damage information into mitogen production. To achieve this objective, we performed fly midgut RNAseq gene expression profile either in healthy tissue or after gut epithelial injury (specifically, bacterial infection, heat shock, oxidative stress, induction of apoptosis, and JNK-mediated stress). Interestingly, RNAseq analysis showed a common set of upregulated gene comprises not only the well-known mitogens Upd2, Upd3 and Vn, but also 40 genes with unknown function in the midgut. Subsequent targeted RNAi-based screen revealed the cell non- autonomous requirement for 6 novel genes in triggering ISC response to bacteria infection. Currently we aim to dissect the function of these genes in the proliferative response upon injury. Understanding how an epithelium senses stressed cells and thereby regulates stem cell proliferation will provide insights into the basis of tissue homeostasis and regeneration.

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Septate Junction Proteins are Required in the Testis Niche for Stem Cell Differentiation. Cameron Berry, Jaclyn Lim, Margaret Fuller. Stanford University, Menlo Park, CA.

The local microenvironment provided by the stem cell niche plays critical roles in regulating the proper self-renewal, proliferation, and differentiation of adult stem cells**.** Current paradigms suggest that these microenvironments provide short-range signals that maintain an undifferentiated state in adult stem cells, so that the daughters of stem cell divisions that are displaced from the niche undergo differentiation by default. However, recent data suggest that this model is too simplistic and that the surrounding microenvironment may also be critical for *promoting* differentiation of the daughters of stem cell divisions. We have identified the non-autonomous requirement of Nrx-IV in the cyst cell lineage to allow for germ cells to progress past the two cell stage. When Nrx-IV is lost in cyst cells via cell type-specific RNAi in adult flies the daughter cells of germline stem cell divisions do not progress through the transit amplifying division, arresting at the two cell stage and undergoing cell death. Nrx-IV is a core component of the invertebrate occluding junction, the septate junction. Additional septate junction components: coracle, pickle/megatrachea, sinuous, lachesin, and dlg were similarly required in cyst cells for germ cell differentiation. Through analysis of endogenous GFP protein trap lines and Immunofluorescence staining, we found that Nrx-IV, ATP-alpha, Nrv2, Nrg, and Dlg were present in the cyst cell lineage and localize to a circumferential ring around differentiating germline cysts. Taken together, this data suggests that the septate junction plays a critical role in the cyst cells for progression of germline cysts through the transit amplifying stage.

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The tumor suppressor homolog Rbf maintains hub cell quiescence and identity, and prevents ectopic niche formation. Leah Greenspan, Erika Matunis. Department of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD, 21205.

Quiescence, or the reversible exit from the cell cycle, is common to most cells in adult tissues but its regulation is not well understood. The *Drosophila* testis provides a unique system to study the regulation of quiescence within an intact stem cell microenvironment, or niche. The testis niche consists of a cluster of 10-15 quiescent somatic cells that signal to adjacent germline and somatic stem cells. Previously we found that forcing hub cells to enter the cell cycle by overexpression of CyclinD/CDK4 induces hub cell divisions and causes a subset of hub cells to transdifferentiate into somatic stem cells. This change in cell fate is accompanied by the formation of ectopic niches, each supporting active stem cells, which causes widespread tissue disruption over time. We have now identified the cell cycle regulator and tumor suppressor retinoblastoma homolog Rbf as a critical regulator of hub cell quiescence. Loss of Rbf in the hub is sufficient to cause hub cell proliferation, transdifferentiation into CySCs, and ectopic niche formation. Continuous as well as transient knockdown of Rbf promotes this uncharacteristic behavior, suggesting that cells lacking this cell cycle inhibitor for even a short time cannot readily return to quiescence. Live imaging of testes under loss of quiescence conditions allows cell fate changes to be tracked in real time and has revealed that transdifferentiating hub cells migrate farther distances than their wild-type counterparts. In contrast, loss of the transcription factor escargot (esg) in the hub causes hub cells to transdifferentiate into CySCs (Voog et al., Cell Reports, 2014), but we have found that this cell fate conversion does not cause ectopic niches to form. We conclude that hub cells are actively kept within a quiescent state and that Rbf is continuously required and may act upstream of esg to maintain these terminally differentiated cells. Our work provides a model for understanding the regulation of cellular quiescence and identity, which could be pertinent in other stem cell niches to prevent over-proliferation and metastases. .

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A somatic permeability barrier around the germline is essential for *Drosophila* spermatogenesis. Michael Fairchild, Christopher Smendziuk, Guy Tanentzapf. Cell and Developmental Biology, University of British Columbia, Vancouver, British Columbia, Canada.

Interactions between the soma and germline are essential for gametogenesis. In the *Drosophila* testis the association of soma and germline is established at the outset of spermatogenesis when differentiating germ cells are encapsulated by two somatic cells. In mammalian systems it is known that the soma forms a tight seal around the germline, known as the Blood-Testis-Barrier, that insulates it from the outside environment. We asked whether the soma in the fly testis performs a similar function in insulating the germline from the outside environment. To this end we developed a novel permeability assay using a fluorescent dye added to media surrounding *ex vivo* cultured testes. Surprisingly this assay showed that encapsulation, by itself, is insufficient for the formation of a permeability barrier around the germline. Instead the encapsulating somatic cells progressively form a permeability barrier around differentiating germ cells during early spermatogenesis. Thus germline stem cells and early germ cells are accessible to the outside environment, while later stages are not accessible as they are isolated by the occluding somatic cells. Furthermore, we find that concomitant with formation of the permeability barrier, septate junction markers are expressed in the soma and localize to the junctional sites connecting the two somatic cells that surround the germline. Moreover, knockdown of septate junction components disrupts both the formation of the permeability barrier, and the differentiation of the germline. Our evidence suggests that this disruption of germline differentiation is due an increase in the signaling range of stem cell maintenance factors that emanate from the stem cell niche. We propose that the somatic permeability barrier around the germline serves an important regulatory function by shaping the signaling environment that the germline experiences during spermatogenesis. Our results identify an important role for the soma during spermatogenesis in the fly and establish a useful model in which to study the establishment and function of the Blood-Testis-Barrier.

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***c-Fos* targeting by the Piwi-piRNA pathway regulates *Drosophila* ovarian germline. Jonathon D. Klein**[1], Chunxu Qu[2], Chunlao Tang[2], Jamy C. Peng[1]. 1) Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN; 2) Computational Biology, St. Jude Children's Research Hospital, Memphis, TN.

The germline tissue requires (i) germ cells to produce gametes and contribute genetic material to the offspring and (ii) somatic cells to form tissues to house and enable gamete production. The *Drosophila*ovary require both germline (GSC) and somatic stem cells (SSC) for oogenesis and provides a unique model to study the interplay of the two populations. GSC function requires Piwi activity in the GSC and the somatic niche. Piwi proteins associate with a class of small RNAs, Piwi-interacting RNAs (piRNAs), to execute their molecular activities. While piRNAs' role in suppressing transposon activity in the germline has been well studied, their possible involvement in Piwi-mediated developmental regulation has been largely unexplored.

We showed that c-Fos, a classic proto-oncogene regulating many cell and developmental processes, is involved in Piwi-mediated regulation of GSC. Reduction of c-Fos, by ways of heterozygous mutations or shRNA-mediated knockdown, rescues *piwi* phenotypes in *Drosophila* ovarian germline. RNA-Seq and IF imaging found Piwi suppresses c-Fos; bioinformatic analysis revealed this likely occurs via the Piwi-piRNA pathway targeting the c-Fos 3' untranslated region (UTR). We used a GFP-reporter assay to confirm that the c-Fos 3'UTR alone is sufficient for gene suppression and that additional piRNAs are generated. Thus, Piwi and piRNAs regulates c-Fos levels by piRNA biogenesis from the 3'UTR of c-Fos mRNA.

To interrogate c-Fos function in oogenesis, we disrupted Piwi-piRNA suppression of c-Fos by replacing its 3'UTR with the K10 UTR. This results in ovarian somatic cell disorganization, tissue dysmorphogenesis, oocyte maturation arrest, and complete infertility. We identified c-Fos as part of the Piwi-mediated regulation of GSC maintenance and differentiation as well as a target of the Piwi-piRNA pathway to regulate SSC and somatic cell organization. These data expand our understanding of the role of non-coding RNAs in

coordinating stem cell populations and tissue morphogenesis.

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Role of non-coding RNAs in stem cell regulation. Megha Ghildiyal[1,3], Blaise Li[2], Hervé Seitz[2], Allan Spradling[1,3]. 1) Department of Embryology, Carnegie Institution for Science, Baltimore, MD, 21210, USA; 2) Institute of Human Genetics, CNRS UPR 1142, Montpellier, France; 3) Howard Hughes Medical Institute.

How do cells establish and maintain an undifferentiated state and then reprogram their chromatin during the process of differentiation? In recent years, evidence has grown that non-coding RNAs play a role in stem cell maintenance and cellular differentiation. In *Drosophila* ovaries, each germ line stem cell (GSC) divides to form a new stem cell and a cystoblast that subsequently divides and differentiates to form an oocyte-bearing-cyst. Stem cell self-renewal reflects both the intrinsic genetic program of the stem cell and the specialized microenvironment in which the stem cells reside, the "stem-cell niche". Several non-coding RNAs have been implicated in maintaining a stem cell state in several cell types (Flynn et al., 2014). Moreover, small RNAs, like piRNAs are essential for germ line development, and evidence suggests a role for small RNAs in the division and the self-renewal of stem cells (Förstemann et al., 2005, Hatfield et al., 2005, Juliano et al., 2011).

To identify candidate noncoding RNAs, we purified GSCs by FACs sorting from *bag of marbles* (*bam*) mutant ovaries that produce tumors of cells very similar to GSCs (Kai et al., 2005). We were able to profile both non-coding and small RNA species in GSCs, and compare them to non-coding RNAs from cystocytes and meiotic germ cells. We are currently trying to define the roles that changing populations of small RNAs play in conferring the GSC's unique identity and in the distinctive events of germ cell development. Of particular interest, is whether subsets of these RNAs mediate specific chromatin changes that occur during this period in female germ cells.

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Genome wide transcriptional analysis of *Drosophila* larvae towards entomopathogenic nematodes. Md. Badrul Arefin[1], Lucie Kučerová[1], Pavel Dobeš[2], Pavel Hyršl[2], Michal Žurovec[3], Ulrich Theopold[1]. 1) Department of Molecular Biosciences, The Wenner-Gren Institute, University of Stockholm, 10691 Stockholm, Sweden; 2) Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; 3) Biology Centre of the AS CR, Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic.

Insect defenses against bacteria and fungi are relatively well understood compared to other pathogens, for instance, entomopathogenic nematodes (EPN). *Heterorhabditis bacteriophora* are entomopathogenic and parasitic worms that contain obligate endosymbionts *Photorhabdus luminescens*. Nematodes invade the host via natural openings or by penetrating the cuticle. We provide for the first time the genome-wide transcriptional analysis of *Drosophila* larvae infected with EPN to elucidate their interactions. While comparing transcriptome profiles between infected and non-infected larvae, we found a total of 642 transcripts differentially regulated upon nematode infection. Gene ontology (GO) analysis showed one-quarter immune-related genes out of the top 100 upregulated genes. We employed reverse genetics for candidate genes to identify their function to the response against nematodes. We primarily focused on immune genes for which a function had already been established in other infection models, and on their paralogs for which immune functions were not known. As the immune reactions are mainly mounted by the fat body, the key immune organ in *Drosophila*, we knocked-down candidate genes in this organ. We supplemented this with hemocyte-specific kockdown, since our observation show that hemocytes are recruited into circulation upon EPN infection. Based on mutants and RNAi analysis, we identified a thioester containing complement-like protein (TEP3), a basement membrane component (Glutactin), a recognition protein (GNBP-like-3) and several small peptides which upon loss showed increased susceptibility after nematode infection.

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Investigating the immune function of the *Drosophila melanogaster* MACPF protein Torso-like. L.J. Forbes Beadle[1], T. Crossman[1], J.C. Whisstock[2], C.G. Warr[1]. 1) School of Biological Sciences, Monash University, Clayton VIC 3800 Australia; 2) Department of Biochemistry and Molecular Biology, Monash University, Clayton VIC 3800 Australia.

Membrane attack complex/perforin-like (MACPF) proteins perform key roles in vertebrate innate immunity. Torso-like (Tsl) is the only known MACPF protein in *Drosophila melanogaster*, discovered for its developmental role in terminal patterning of the embryo. We have discovered an additional role for Tsl in the *Drosophila* immune response and aim to determine how a MACPF protein such as Tsl can function in both development and immunity. We have shown that *tsl* null mutants have an increased susceptibility to infection by gram-positive, but not gram-negative bacteria. This contrasts with the role of the mammalian membrane attack complex, which specifically targets gram-negative bacteria through disruption of the outer bacterial membrane. Interestingly, despite this increased susceptibility, upon infection *tsl* null mutants show normal induction of the transcriptional targets *Drosomycin* and *Diptericin* of the Toll and Imd systemic pathways. As some phagocytic mutants (eg. *eater* null, Nehme *et al.*2011) show an increased susceptibility to bacterial infection similar to that seen in *tsl* mutants, we hypothesised that Tsl may be required for cellular immunity. We found that *tsl* mutants showed a significant reduction in their phagocytic ability. We are currently exploring the hypothesis that Tsl is required for membrane disruption in both development and immunity.

Nehme NT, Quintin J, Cho JH, Lee J, Lafarge M-C, et al. (2011) Relative Roles of the Cellular and Humoral Responses in the *Drosophila* Host Defense against Three Gram-Positive Bacterial Infections. PLoS ONE 6(3): e14743.

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The acetate switch of an intestinal pathogen disrupts host insulin signaling and lipid metabolism. S Hang[1], A Purdy[1], W Robins[2], Z Wang[1], M Mandal[1], S Chang[1], J Mekalanos[2], P Watnick[1]. 1) Division of infectious Diseases, Boston Children's Hospital, Boston, MA; 2) Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA.

Ingestion of *Vibrio cholerae* is lethal to the model host *Drosophila melanogaster*. A genetic screen for bacterial virulence factors in this model identified CrbRS, a previously undefined two-component system known to be active in the human intestine. Here we show that, through transcriptional activation of the gene encoding acetyl-CoA synthase-1 (ACS-1), CrbRS controls the *V. cholerae* acetate switch, in which cells transition from excretion to assimilation of environmental acetate. We provide evidence that the absence of intestinal acetate leads to deactivation of host insulin signaling and accumulation of lipid droplets in enterocytes. Short chain fatty acids (SCFA) such as acetate, which are supplied by commensal intestinal bacteria, are essential for organismal health. Our findings suggest a novel virulence mechanism in which consumption of these SCFA by an intestinal pathogen enhances initial colonization of the intestine, deactivates host insulin signaling, and leads to dramatic and lethal dysregulation of host lipid transport. .

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Armadillo modulates intracellular titers of *Wolbachia* bacteria in *Drosophila* gonads. Ajit Kamath, Michelle Toomey, Rama Krishna Simhadri, Horacio Frydman. Biology, Boston University, Boston, MA.

Wolbachia are maternally transmitted intracellular bacteria infecting up to 40% of insect species, including most *Drosophila* species. Introduction of *Wolbachia* from *Drosophila* into mosquitoes increase their resistance to human pathogens, including Dengue, West Nile Virus and Plasmodium. *Wolbachia* tissue tropism and titers have been implicated in pathogen blockage in mosquitos. However, our understanding of the molecular mechanisms of *Wolbachia* tissue tropism and intracellular accumulation is limited.
The *Drosophila* gonads provide an ideal system to address this question, as *Wolbachia* have tropism for specific cell types in the gonads: the stem cell niches in males and females and the polar cells in the ovaries. We undertook a candidate gene approach to study the effect of highly expressed proteins within these cell types on *Wolbachia* density. In this study, we report that wnt/wg signaling via Armadillo is responsible for modulating *Wolbachia* levels in somatic cells in the *Drosophila* gonads. RNAi-mediated knockdown of armadillo in the polar cells (ovary) and hub (testis) resulted in a significant decrease in *Wolbachia* density. Armadillo is a dual-function protein with a crucial role in wnt/wg signaling as well as in adherens junctions mediating cell-cell adhesion. To assess the contribution of adherens junctions on *Wolbachia* levels, we knocked down DE-Cadherin. However this had no significant effect on *Wolbachia* densities suggesting that Armadillo-mediated regulation of *Wolbachia* density is independent of the adherens junctions and that Armadillo's role in the wnt/wg signaling is modulating*Wolbachia* density in various host tissues. To further validate this hypothesis, we expressed a constitutively active form of Armadillo that activates wnt signaling. Confirming our hypothesis, when wnt signaling was activated, we observed a significant increase in *Wolbachia* density. This study implicates a direct effect of a host signaling pathway on intracellular symbiont densities. Examining additional host proteins and signaling molecules will provide insights into the complex interplay between intracellular symbionts and host proteins.

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Preventing age-related metaplasia promotes homeostasis of the gastrointestinal tract and extends lifespan. Hongjie Li[1,2], Yanyan Qi[1], Heinrich Jasper[1]. 1) Buck Institute for Research on Aging, Novato, CA; 2) University of Rochester, Biology Department, Rochester, NY.

Intestinal metaplasias, i.e. transformation of regionally specialized epithelia into ectopic epithelial subtypes, are associated with aging and considered a risk factor for gastrointestinal cancers. The cellular origin of the metaplastic tissue and underlying molecular mechanisms driving the epithelial transformation are poorly understood to date. The *Drosophila* gastrointestinal (GI) tract, which is lined by a series of functionally specified epithelia, has emerged as an important tool to explore the regional diversification of epithelial regeneration and the maintenance of epithelial subtypes. The fly GI tract consists of anterior midgut (AM), middle midgut (MM) and posterior midgut (PM). The MM contains an acidic stomach-like copper cell region (CCR). Maintenance of specific epithelia within these regions is critical to maintain GI function and homeostasis. Our previous work has shown that BMP/Dpp signaling determines and maintains the identity of gastric stem cells, allowing long-term regeneration of acid-producing copper cells. Here we show that the maintenance of the CCR declines with age due to the metaplastic replacement of copper cells by cells expressing markers specific for AM or PM epithelia. This results in the age-related decline of pH homeostasis of the GI tract as well as commensal dysbiosis and epithelial dysplasia in other regions of the gut. We show that the CCR metaplasia is caused by age-related activation of stress signaling, most likely due to systemic inflammation, and that this constitutive activation inhibits BMP/Dpp mediated regeneration and maintenance of copper cells. Accordingly, inhibiting the identified stress signaling in the CCR specifically is sufficient to rescue the age-related metaplasia, to promote gut function, and to extend lifespan.

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Regulation of metabolism and insulin sensitivity by Sir2 in *Drosophila*. Rebecca A.S. Palu, Carl S. Thummel. Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Complex metabolic disorders such as obesity and diabetes are on the rise in developed countries worldwide, highlighting the importance of characterizing the factors involved in their development and progression. In large part, the complexity of these disorders arises from interactions between genetic and environmentally induced pathways that determine individual metabolic health. The highly conserved deacetylase Sir2 (Sirt1) plays an important role in integrating these pathways due to its widespread effects on protein

acetylation state in response to levels of NAD^+, an important electron carrier in central metabolic pathways. Consistent with this, loss of *Sirt1* is associated with metabolic dysfunction and diabetes in mice and humans, although its molecular roles in these pathways remain unclear. Previous work has demonstrated that deletion of *sir2* in *Drosophila* leads to increased triglyceride levels at both adult and larval stages. We have confirmed this effect and shown that *sir2* mutants are also hyperglycemic under both fed and fasted conditions, a hallmark of diabetes. These mutants become increasingly glucose intolerant with time, which is associated with a concurrent loss of peripheral insulin signaling and insulin resistance. Tissue-specific RNAi and rescue experiments demonstrate that Sir2 functions in the fat body, indicating a role for Sir2 in regulating insulin sensitivity as opposed to secretion. Evidence from metabolomics analysis supports this hypothesis and suggests that there is altered flux through glycolysis and the TCA cycle. Additionally, RNA-seq transcriptional profiling reveals significant overlaps between Sir2-regulated genes and those regulated by metabolic transcription factors, including HNF4, FOXO, and HR96, raising the possibility that Sir2 directly deacetylates these factors. Taken together, we have shown that loss of *sir2* is a genetic model for insulin resistance and subsequent diabetes in *Drosophila*. Our current focus is to further characterize the molecular mechanisms of Sir2 action and identify its direct targets.

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Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist *ImpL2*. Young Kwon[1], Wei Song[1], Ilia Droujinine[1], Yanhui Hu[1], John Asara[3,4], Norbert Perrimon[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA; 3) Department of Medicine, Harvard Medical School, Boston, MA; 4) Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA.

Organ wasting, related to changes in nutrition and metabolic activity of cells and tissues, is observed under conditions of starvation and in the context of diseases such as advanced cancers. We have developed a model for organ wasting in adult *Drosophila*, whereby overproliferation induced by activation of Yorkie, the Yap1 oncogene ortholog, in intestinal stem cells, leads to wasting of the ovary, fat body and muscle tissues. Metabolic analyses reveal that these organ-wasting phenotypes are associated with a reduction in systemic insulin/IGF signaling due to increased expression of the secreted insulin/IGF antagonist *ImpL2* from the overproliferating gut. Strikingly, expression of rate-limiting glycolytic enzymes and central components of the insulin/IGF pathway is up-regulated with activation of Yorkie in the gut, which may provide a mechanism for this overproliferating tissue to evade the effect of ImpL2. Altogether, our study provides insights into the mechanisms underlying organ-wasting phenotypes in *Drosophila* and how overproliferating tissues adapt to global changes in metabolism.

157

Mechanism of Body Fat Regulation by Split ends. Kelsey Jensen, Tânia Reis. Department of Medicine, University of Colorado Medical School, Aurora, CO.

Genetic background plays a major role in obesity. However, only a few of the responsible genes have been identified. We previously identified sixty-six *Drosophila* genes that increase body fat when mutated. Here we present our analysis of one of these, *split ends (spen)*. Spen is a transcriptional regulator with known roles in multiple pathways, previously implicated in neuronal development. Spen is also expressed in the larval fat body (FB), and FB-restricted manipulation of Spen levels by RNAi or overexpression increases or decreases larval body fat, respectively. We further find that in addition to this FB-autonomous fat-regulatory role, *spen* regulates organismal fat non-autonomously via metabolic behaviors, including food intake and activity. Interestingly, the high-fat phenotype of larvae in which Spen is depleted from the FB is accompanied by starvation sensitivity, suggesting that Spen regulates pathways that promote utilization of stored energy. In order to identify conserved fat-regulatory roles for Spen, we assessed levels of its mouse ortholog, MINT, in a diet-induced obesity model, and find that MINT is upregulated in the liver of obese mice compared to their sibling lean controls. Currently, we are dissecting the mechanistic details of Spen's function as a candidate conserved anti-obesity regulator.

158A

Quantification of mechanical force driving left-right asymmetric morphogenesis of the embryonic gut in *Drosophila*. Mai Adachi[1], Naotaka Nakazawa[2], Shukei Sugita[3], Takeo Matsumoto[3], Kenji Matsuno[1]. 1) Osaka University, Osaka, Japan; 2) National University of Singapore, Lower Kent Ridge Road ,Singapore; 3) Nagoya Inst. of Tech, Aichi, Japan.

In *Drosophila*, many internal organs show directional LR asymmetry. The LR asymmetric structure of the embryonic hindgut is formed through a 90 degree counterclockwise rotation by the mechanical force generated by the hindgut itself. Before this rotation, the shape of hindgut epithelial cells at the apical plane is LR asymmetric in *vivo*. Given that their three dimensional structure does not overlap with that of their mirror image, this LR asymmetric property is referred to as "planar cell chirality" (PCC). Our computer modeling suggests that the PCC is responsible for this left-handed rotation.

To explore the genetic pathway that generates mechanical force inducing the rotation of the hindgut, we need to analyze the force quantitatively. We developed a novel method to measure the torque of the hindgut counterclockwise rotation using the magnetic beads. Before the rotation of the embryonic hindgut, its anterior end curves towards the ventral side. Magnetic beads were microinjected into the lumen of the hindgut anterior end. Using neodymium magnets, we induced counterbalancing magnetic force against the twisting of the hindgut. As a reference of how much force is created, we measure the magnetic force between the magnetic beads and the neodymium magnet in viscous fluid. We successfully quantified the magnetic force required to stop the contortion of the hindgut, which is equivalent to the force generated by the hindgut for its twisting. These values are crucial for performing quantitative genetic analysis of mechanical force inducing the LR asymmetric morphogenesis.

159B

Differential roles of the unconventional Dock family members Myoblast city and Sponge in Drosophila development. Bridget H Biersmith[1], Erika R Geisbrecht[2]. 1) University of Missouri - Kansas City, Kansas City, Missouri, MO; 2) Kansas State University, Manhattan, KS.

The evolutionarily conserved Dock proteins function as unconventional guanine nucleotide exchange factors (GEFs). Upon binding to ELMO (Engulfment and cell motility) proteins, Dock-ELMO complexes activate the Rho family of small GTPases to mediate a diverse array of biological processes, including cell motility, apoptotic cell clearance, and axon guidance. Overlapping expression patterns and functional redundancy among the eleven vertebrate Dock family members, which are subdivided into four families (Dock-A, B, C, and D), complicate genetic analysis. Both in vertebrate and invertebrate systems, the actin dynamics regulator, Rac, is the target GTPase of the Dock-A subfamily. However, it remains unclear whether Rac or Rap1 are the in vivo downstream GTPases of the Dock-B subfamily.*Drosophila melanogaster* is an excellent genetic model organism to understand Dock protein function as its genome encodes one ortholog per subfamily: Myoblast city (Mbc; Dock-A) and Sponge (Spg; Dock-B). Herein we show that the roles of Spg and Mbc are not redundant in the *Drosophila* somatic muscle or the dorsal vessel (dv). Moreover, we confirm the *in vivo* role of Mbc upstream of Rac and provide evidence that Spg functions in concert with Rap1 to regulate aspects of cell adhesion. Together these data show that Mbc and Spg can have different downstream GTPase targets. Our findings predict that the ability to regulate downstream GTPases is dependent on cellular context and allows for the fine-tuning of actin cytoskeletal or cell adhesion events in biological processes that undergo cell morphogenesis.

160C

Defining the role of CG1674 in adult muscle development. Marilyn Cisneros, Richard Cripps. Biology, University of New Mexico, Albuquerque, NM.

In *Drosophila*, the study of muscle formation and structure can provide a better understanding of the factors that trigger muscle disease in humans. Our current goal is to determine if the protein CG1674 is a component of the sarcomere and if it is required for muscle structure and function. CG1674 was discovered through proteome sequencing of dissected flight muscles. This sequencing data suggests that this protein is a functional component of the flight muscles of *Drosophila*. We are currently generating a CG1674-GFP fusion protein to determine the localization of our protein within the muscle cell. In parallel, we are determining the requirement for CG1674 in normal muscle formation by expressing in the flight muscles an RNAi targeting the *CG1674* transcript. When crossing *UAS-CG1674 RNAi* with the drivers *Mef2Gal4* and *1151Gal4*, the flies are flightless. Immunohistochemical staining of the muscles reveals defects in myofibril formation and structure. These studies identify a potentially novel muscle protein that is required for normal muscle assembly and function.

161A

Compound Heterozygosity of Meckel-Gruber Syndrome Proteins. Nicole M Clark, Marcus L Basiri, Andrew Ha, Avidor-Reiss Tomer. Biological Sciences, University of Toledo, Toledo, OH.

Cilia are important sensory and motility organelles found in most animal cells. Mutations in genes associated with the cilium are known to cause many lethal diseases in humans, collectively referred to as ciliopathies (e.g. Meckel-Gruber syndrome, Bardet-Biedl syndrome, and Nephronophthisis). In general, these genetic diseases exhibit autosomal recessive inheritance. Using the fly, Drosophila melanogaster, as a model organism, we have results suggesting a ciliary defect originating from compound heterozygosity. In other words, this ciliary defect may be the consequence of two mutations, each in a distinct gene.
The Meckel-Gruber syndrome proteins Cep290 and B9D1 are essential for ciliary formation and function. Homozygote flies of *cep290* exhibit a severe *unc*-type uncoordination (in which the fly is unable to walk and has erect wings) and sperm immotility. We have identified a severe loss of function mutation of *b9d1* that exhibits a similar phenotype. To our surprise, we found that compound heterozygotes of*cep290* and *b9d1* also exhibit the same phenotypes. Additionally, hemizygotes with either mutant *cep290* or *b9d1* over the deficiency of the other gene demonstrate the same uncoordination. This phenotype may be due to the combination of *cep290* and *b9d1* mutations. However, it is possible that additional mutations in other undefined genes contribute to these phenotypes. To distinguish between the role of a compound heterozygote and that of additional genes, we use meiotic mapping to identify the location of the genes underlying the above phenotypes. .

162B

Pavarotti/MKLP1 regulates microtubule sliding and neurite outgrowth in *Drosophila* neurons. U. del Castillo, W. Lu, M. Winding, M. Lakonishok, V. Gelfand. Feinberg School of Medicine, Cell and Molecular Biology Dept, Northwestern University, Chicago, IL.

Recently, we demonstrated that kinesin-1 can slide microtubules against each other providing the mechanical force required for initial neurite extension in *Drosophila* neurons. This sliding is only observed in young neurons actively forming neurites and is dramatically downregulated in older neurons. The downregulation is not caused by the global shut-down of kinesin-1, as the ability of kinesin-1 to transport membrane organelles is not diminished in mature neurons, suggesting that microtubule sliding is regulated by a dedicated mechanism. Here, we have identified the “mitotic” kinesin Pavarotti (Pav-KLP) as an inhibitor of kinesin-1-driven microtubule sliding. Depletion of Pav-KLP in neurons strongly stimulated the sliding of long microtubules and neurite outgrowth, while its ectopic overexpression in the cytoplasm blocked both of these processes. Furthermore, postmitotic depletion of Pav-KLP in *Drosophila* neurons *in vivo* reduced embryonic/larval viability, with only a few animals surviving to the third instar larval stage. A detailed examination of motor neurons in the surviving larvae revealed the overextension of axons and mistargeting of neuromuscular

junctions, resulting in uncoordinated locomotion. Taken together, our results identify a new role for Pav-KLP as a negative regulator of kinesin-1 driven neurite formation. These data suggest an important parallel between long microtubule-microtubule sliding in anaphase B and sliding of interphase microtubules during neurite formation.

163C

Branched F-actin dynamics regulate contact length during Drosophila eye morphogenesis. Steven Del Signore, Victor Hatini. Dev, Mol, & Chem Biology, Tufts Univ Sch Med, Boston, MA.

During development, epithelial cells must both generate and respond to mechanical forces to elaborate morphologically and functionally differentiated tissues. During morphogenetic events such as gastrulation and germ band extension, contractile forces generated by the F-actin-Myosin II (actomyosin) cytoskeleton constrict and eliminate cell contacts to promote apical constriction and cell intercalation, respectively. However, the mechanisms that counteract contractile forces to prevent contact elimination or to promote contact expansion during morphogenesis remain poorly understood. To investigate this question, we examined late stages of *Drosophila* eye morphogenesis, during which cells dramatically change shape but maintain cell-cell contacts. By live imaging of MyoII, F-actin, and cell contact dynamics, we found that shrinking cell-cell contacts constricted in an oscillatory manner, with phases of constriction followed by phases of transient contact expansion. Importantly, these phases correlated with contractile actomyosin and protrusive F-actin dynamics, respectively. As the role of protrusive F-actin dynamics in epithelial morphogenetic processes has not been studied, we further analyzed these dynamics. We hypothesized that the observed F-actin dynamics are composed of branched F-actin, which is generated by the activities of the Arp2/3 complex and its upstream activator, the WAVE homolog *SCAR*. By live imaging of Arp3 and F-actin, we found that F-actin correlated spatiotemporally with Arp2/3 complex dynamics. Supporting a functional role for protrusive F-actin dynamics to oppose contractile forces, we found that SCAR enriched at dynamic contacts, and that *SCAR* mutant clones exhibited constricted apical surfaces and abnormal cell contacts. To test whether these dynamics act to balance contractile forces, we experimentally enhanced contractile forces through expression of the linear F-actin regulator Diaphanous, and found that characteristics of both morphological and molecular oscillations were altered. Together, these data suggest a mechanism by which contractile and protrusive forces coordinate at cell-cell contacts to robustly regulate contact length, cell shape, and tissue topology.

164A

Dynamic analysis of the function of the actin cable during *Drosophila* dorsal closure. Antoine Ducuing[1], Maxime Dureau[1], Charlotte Keeley[2], Bertrand Mollereau[1], Stéphane Vincent[1]. 1) Laboratory of Molecular Biology of the Cell, Ecole Normale Supérieure de Lyon, Lyon, France; 2) Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.

Dorsal closure (DC) is a key morphogenetic process where hundreds of leading edge (LE) cells differentiate and act in concert to seal the dorsal opening covered by the Amnioserosa (AS). Specifically, LE cells produce a supra-cellular acto-myosin cable (AC) that circles the AS. LE cells also produce dynamic, actin-rich protrusions called filopodia. While the filopodia are crucial for the final step of cell matching, the precise function of the AC remains elusive. The AC could provide a contractile force and act as a purse-string mechanism, or could prevent the ventral-ward relaxation of leading edge cells, thus acting as a ratchet. Still, the precise contribution of the AC during closure remains unclear since no studies have reported the analysis of a mutant lacking solely the cable. Here we show that during dorsal closure Zasp52, an actin-associated protein is enriched at the actin cable and in filopodias. Zasp52 mutant embryos display defects in the LE straightness during DC, suggesting that the AC organisation is affected. Specifically, we found that the accumulation of both Actin and the actin-capping molecule Enabled at the LE/AS interface is lost in Zasp52 mutant embryos, confirming that these embryos are deprived of an AC. In addition, cell-junction cut experiments at the AS/LE interface show that Zasp mutant embryos lack tension. Interestingly, filopodia are still detected and microtubules polarized along the dorso-ventral axis still accumulate. Altogether, Zasp52 mutant embryos provide the perfect system to assess the precise contribution of the actin cable to dorsal closure. High-throughput live imaging reveals that closure proceeds 50% slower in Zasp mutant embryos, suggesting that the actin cable is dispensable for DC. In order to understand how the cable improves closure efficiency we used high-frequency live imaging combined with segmented image analysis. We will present our results based on this multidisciplinary approach.

165B

Integrins regulate apical constriction via microtubule stabilization in the *Drosophila* eye disc epithelium. Vilaiwan M. Fernandes[1], Kasandra McCormack[1], Lindsay Lewellyn[2], Esther M. Verheyen[1]. 1) Simon Fraser University, Burnaby, BC, Canada; 2) Butler University, Indianapolis, Indiana, USA.

During morphogenesis, extracellular signals trigger actomyosin contractility in subpopulations of cells to coordinate changes in cell shape. To illuminate the link between signaling-mediated tissue patterning and cytoskeletal remodeling, we study the progression of the morphogenetic furrow (MF), the wave of apical constriction that traverses the *Drosophila* eye imaginal disc preceding photoreceptor neurogenesis. Apical constriction depends on actomyosin contractility downstream of the Hedgehog (Hh) and BMP pathways. We identify a novel role for integrin adhesion receptors in MF progression. We show that Hh and BMP regulate integrin expression, the loss of which disrupts apical constriction and slows furrow progression; conversely, elevated integrins accelerate furrow progression. We present evidence that integrins regulate MF progression by promoting microtubule stabilization since reducing microtubule stability rescues integrin-mediated furrow acceleration. Thus integrins act as a genetic link between tissue-level signaling events and morphological change at the cellular level, leading to morphogenesis and neurogenesis in the eye.

166C

Clic functions to regulate Moesin phosphorylation during *Drosophila* rhabdomere formation. Kara J Finley[1], Robert Hikida[2], Mark Berryman[2], Soichi Tanda[1]. 1) Department of Biological Sciences, Ohio University, Athens, OH; 2) Department of Biomedical Sciences, Ohio University, Athens, OH.

ERM family proteins have a critical role in the formation of actin-rich structures through the process of linking the cytoskeleton to the plasma membrane. In *Drosophila melanogaster*, Moesin, the sole ERM protein, is responsible for the development of the stacks of thousands of actin-based microvilli that form the rhabdomeres of photoreceptors in the compound eye. The phosphoregulation of Moesin is thought to be accomplished through the activities of various proteins. Moesin binds to the phospholipid PIP_2, which is produced through the action of Sktl. Slik kinase then phosphorylates Moesin, thereby activating it and promoting binding of the actin cytoskeleton. This Moesin-actin complex is transferred from PIP_2 to a membrane-bound protein via the protein Sip1 (*Drosophila* homolog of EBP50). Dephosphorylation of Moesin can then occur, leading to separation of the actin cytoskeleton from the plasma membrane. The dephosphorylated, inactive form of Moesin can reenter this cycle by binding to PIP_2. The chloride intracellular channel protein Clic is implicated to have a role in this pathway. Our study focuses on determining the function and location of Clic in the Moesin phosphoregulation cycle. We overexpressed Slik, both individually and in combination with Sip1 or Sktl, with a GMR driver. We compared these in the wild type and a Clic deficient background. Offspring were compared by external morphology and internal eye morphology using a transmission electron microscope. Excess Slik kinase or Sktl, and the combination of both increases the phosphorylation of Moesin, thereby leading to larger and more irregular rhabdomere formations; loss of Clic function enhances these effects. Loss of Clic appears to result in a slight rescue of the phenotype of offspring with excess Slik kinase and Sip1, leading to a more regular rhabdomere organization. These results suggest Clic functions to transfer phosphorylated Moesin to membrane-bound proteins, possibly with Sip1, promoting the dephosphorylation of phosphorylated Moesin for recycling.

167A

Biochemical optimization of the purification of the C-terminal domains of *ck*/MyoVIIA. Thomas Halverson, Jennifer Sallee. Science, North Central College, Naperville, IL.

Myosin VIIA (MyoVIIA) is an actin-based motor protein that is found in both *Drosophila melanogaster* and humans, and is necessary for the formation of actin-based protrusions. Mutations in human MyoVIIA result in deafness due to defects in the actin-based stereocilia (Usher Syndrome) while in *Drosophila*, mutants of *ck*/MyoVIIA are also deaf and display defects in actin-based structures like bristles and denticles. In order to determine the molecular mechanism behind *ck*/MyoVIIA's function we want to identify potential binding partners of *ck*/MyoVIIA in *Drosophila melanogaster*. A transgenic *ck*/MyoVIIA containing the C-terminal tail binding domains and fused to a triple affinity tag (FLAG-His-StrepII) was isolated from adult fly lysate. Initially, we encountered problems keeping the protein soluble while purifying. Therefore, we had to optimize the appropriate buffer conditions to solubilize the protein from fly lysates. To do this we used western blot analysis with a FLAG antibody and compared the amount of soluble proteins between conditions. We discovered that increasing the concentrations of detergents increased the solubility of the protein. Currently, we are optimizing the conditions to purify the protein via it's His tag on a Talon affinity matrix so we can identify possible binding partners.

168B

F-actin turnover is essential for apical constriction and tissue morphogenesis. J. N. Jodoin[1], M. Tworoger[1], L. Perkins[2], N. Perrimon[2,3], A. C. Martin[1]. 1) Biology, MIT, Cambridge, MA; 2) Genetics, Harvard, Boston, MA; 3) HHMI, Harvard, Boston, MA.

Apical constriction is a cell shape change that promotes epithelial bending and results in the invagination of cells within the embryo. In many cases apical constriction is driven by the contraction of an actin filament (F-actin) meshwork by Myosin II (myosin) motors, which generate tension across the apical surface of the epithelia. Apical constriction and apical tension are conventionally thought to be associated with stable F-actin and elevated F-actin levels. The role of F-actin disassembly and the subsequent recycling of actin subunits (turnover) in generating apical tension and epithelial cell shape change is unknown. Here, we used a live-imaging based RNAi screen, to identify a critical role for F-actin disassembly and monomer recycling in maintaining stable intercellular mechanical connections during epithelial bending. Our screen targeted the *Drosophila* Actin-ome consisting of 60 genes screened and a total of ~350 initial movies, which allowed us to identify subtle phenotypes that may have been missed by less sensitive assays involving fixed embryos. We identified at least 6 phenotypic classes, or functional modules, that define functions regulating myosin dynamics, actin network architecture, and the stable transmission of forces between cells throughout the epithelial folding process. Disruption of genes that regulate F-actin disassembly represented one of the strongest phenotypic classes, with the gene *capt*being required for apical constriction and epithelial bending; *capt* depletion results in an F-actin pile-up near the center of the apical surface. Using both *capt* depletion and cytoskeletal inhibitors that slow F-actin disassembly, we show that efficient F-actin disassembly is critical to remodel the apical F-actin network during constriction and to maintain stable mechanical connections between cells. In addition, we identified a separate module including monomer sequestering/recharging proteins, *chic* and *cib*, which do not result in an F-actin build-up, but are also essential to maintain stable mechanical connections between cells. In summary, our work identifies a critical role for F-actin disassembly and subsequent monomer recycling in transmitting tissue-level forces during morphogenesis. .

169C

Identifying a Kelch-Cullin3 ubiquitin ligase substrate. Katelynn Mannix, Andrew Hudson, Lynn Cooley. Department of Genetics, Yale School of Medicine, New Haven, CT.

In the Drosophila egg chamber, ring canals are intercellular bridges composed of a robust actin cytoskeleton. Mutations in *kelch* cause

accumulation of F-actin in the lumen of ring canals. It was previously shown that Kelch is an F-actin cross-linking protein but more recent work suggests that Kelch also functions with Cullin3 (Cul3) at ring canals. Cul3 germline clones show a *kel*-like ring canal phenotype. We hypothesize that Kelch functions as a substrate adaptor protein for a Cullin3-RING ubiquitin E3 ligase (CRL3). CRL3s usually target protein substrates for ubiquitination and degradation by the proteasome. To determine if the proteasome is required for proper ring canal cytoskeleton organization, we inhibited the proteasome in the female germline with shRNA lines (from TRiP) targeting different proteasomal genes. We also developed a proteasome activity reporter to monitor the effectiveness of proteasome inhibition. Indeed, proteasome inhibition leads to a *kel*-like ring canal phenotype. These data suggest that Kelch and Cul3 function together as components of an E3 ubiquitin ligase to target a ring canal protein for ubiquitylation and degradation by the proteasome, a process required for disassembly of the lumenal ring canal cytoskeleton. To identify the CRL3-Kelch substrate, we are employing a new proteomics technique developed by the Ting lab at MIT that uses a targetable APEX enzyme that is capable of biotinylating nearby proteins with great specificity. The APEX enzyme was fused to a ring canal-specific protein (HtsRC) and expressed in egg chambers to target APEX specifically to ring canals, where it can biotinylate ring canal proteins. Biotinylated ring canal proteins were purified and subjected to mass spectrometry (MS) to identify novel ring canal proteins. Currently we are screening through proteins identified by MS to determine if any are candidate CRL3-Kelch substrates. As an alternative approach to identify the CRL3-Kelch substrate at ring canals, we are working to directly immunoprecipitate Kelch from egg chambers in conditions where the CRL3-Kelch-substrate complex should be stabilized and perform MS to identify Kelch-interacting proteins.

170A

Rho GAPs are required for proper organization of the apical actomyosin cortex and epithelial invagination. Frank Mason, Michael Tworoger, Adam Martin. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

During *Drosophila* gastrulation, apical constriction of ventral furrow cells promotes tissue bending and is thought to require the apical activation of the Rho1 GTPase. Prior research on the mechanism of apical constriction has predominantly focused on the role of DRhoGEF2 in promoting apical Rho1 activation. However, in the ventral furrow, there is no known function of Rho1 inhibitors or GTPase activating proteins (GAPs) that could regulate the dynamics or spatial organization of the Rho1 signal during apical constriction. Here, we performed an RNAi screen against all *Drosophila* Rho-family GAPs expressed in the early embryo and identified a GAP that is required for ventral furrow formation. Embryonic depletion of the Rho GAP results in a broader Myo-II distribution across the apical cortex and more irregular Myo-II pulses or temporal dynamics. Finally, Rho GAP depletion results in cell adhesion and ventral furrow invagination defects. Our results demonstrate a previously unrecognized function for Rho GAPs in promoting spatiotemporal organization of the apical cortex and reveal a requirement for restricting Rho1 activity during ventral furrow invagination.

171B

Characterization of *twinfilin* mutants in denticle formation. Jane M. McCullough, Kayla Antle, Jennifer Sallee. North Central College, Naperville, IL.

The formation of actin-based protrusions, such as the denticles in *Drosophila melanogaster*, is a complex process involving the coordination of several actin-associated proteins. Mutations in such proteins can cause defects in the shape, size, and function of actin-based protrusions. Twinfilin, which localizes at sites of actin filament assembly, functions as an actin monomer sequestering protein. Mutations in the *Drosophila* t*winfilin* gene have been found to create bristle defects in adults, but the effects of mutant *twinfilin* on denticle formation remain unclear. In addition, Myosin VIIA is an actin-based motor protein necessary for the formation of multiple actin-based protrusions. Mutations in human Myosin VIIA result in deafness due to defects in the actin-based stereocilia (Usher Syndrome) while in *Drosophila*, *ck*/Myosin VIIA mutants are also deaf and display defects in bristles and denticles. In this study, we examined the effect of twinfilin and *ck/*Myosin VIIA single mutations and twinfilin-Myosin VIIA double mutations on denticle morphology in *Drosophila melanogaster* embryos. Homozygous *twinfilin*^{110} and *twinfilin*^{3701} mutants showed deformed denticles, specifically an increase in unhooked denticles in rows 2 and 4 in comparison to wild type embryos. Myosin VIIA and twinfilin double mutants are currently being examined in order to determine whether there is a genetic interaction between the two genes. .

172C

A coupled *in vitro-in vivo* approach to dissect APC-Diaphanous mediated actin assembly. Olivia Molinar[1], Richa Jaiswal[2], Ezgi Kunttas-Tatli[1], Aneliya Rankova[2], Vince Stepanik[1], Bruce L. Goode[2], Brooke M. McCartney[1]. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA.

The multifunctional Adenomatous polyposis coli (APC) proteins negatively regulate Wnt signaling, stabilize microtubules, and indirectly regulate actin through effectors such as Asef and IQGAP. We have shown that both vertebrate APC (vAPC) and Drosophila APC1 through their basic domains can bundle and nucleate actin filaments, and collaborate with the formin Diaphanous (Dia) to efficiently nucleate actin assembly *in vitro*. In addition, Drosophila APC2 (lacking a basic domain) and Dia bind directly, and are required for actin furrow extension in the embryo. In contrast, APC1 does not function in actin furrow extension. While these data suggest that APC-Dia collaborations are an evolutionary conserved mode of actin filament assembly, significant gaps exist in our understanding of mechanism and physiological relevance. We have investigated the mechanism underlying the vAPC/APC1-Dia collaboration in detail, but how APC2 affects Dia without a basic domain is not known. Here we demonstrate that APC2 interacts with Dia through its ß-catenin binding 20 amino acid repeats (20Rs), and that 20R phosphorylation by GSK3ß regulates APC2's actin furrow activity. Furthermore, we have taken advantage of the many coordinated actin-mediated processes that occur during Drosophila oogenesis to investigate the physiological role of APC1 mediated actin assembly. In stage 10B egg chambers, an array of cytoplasmic actin bundles (actin baskets)

form around each nucleus in the nurse cells to secure them. Here we show that APC1 and Dia are required for the proper assembly of these actin baskets. In *APC1* or *dia* mutants, the actin baskets fail to form at stage 10B, but surprisingly they are present at stage 11. This suggests that APC1 or Dia may be able to carry out actin assembly alone, but with reduced efficiency resulting in assembly delay. Taken together, our studies suggest that APC-Dia collaborations are evolutionarily conserved, and that Drosophila is an experimentally tractable system in which to dissect both molecular mechanisms and physiological relevance. .

173A

Actomyosin network dynamics during epithelial cell alignment. Katy Ong, Stephen DiNardo. Cell and Developmental Biology Department, University of Pennsylvania, Philadelphia, PA.

Coordination of cell shape changes among groups of cells is required for a tissue to adopt its proper morphology, which, in turn, is necessary for proper function. Dysregulated morphogenesis underlies pathologies such as developmental defects and cancer. The lowest energy state of a simple epithelium is a hexagonal packing where cells contact one another at 3-way vertices. However, many epithelia must adopt cell packing geometries far from equilibrium in order to perform their function. Two types of molecular machines are required to generate such geometries: **(1)** force-generating networks to provide the energy input for morphogenesis **(2)** polarity determinants to spatially regulate these networks to achieve the proper cell shape change.
As a model of epithelial morphogenesis, we study a dramatic alignment in the embryonic epidermis that produces columns of rectangular cells. These columns generate lines of denticle hooks required for larval mobility; thus the geometry of this epithelium is key to its function. Past work in our lab has suggested that actomyosin-dependent junction remodeling events produce this rectilinear arrangement by converting 3- to 4-cell vertices. To test this hypothesis, we performed live imaging to quantify cell shape changes during alignment at high temporal resolution. We found that the straightening of dorsal-ventral (DV) oriented contacts between cell columns did not temporally correlate with junction remodeling events. These results suggest that alignment is not a consequence of cell vertex conversion alone, and that additional mechanisms are required to fully explain the morphology of this epithelium. We propose that alignment of the denticle field is driven by tensile force along DV boundaries generated by actomyosin cables. Actomyosin cables co-localize with aligning DV junctions throughout alignment and similar cables have been demonstrated to form smoothened boundaries in several epithelia. In addition, we observe a highly dynamic pool of medial-apical myosin in aligning cells, similar to that observed during ventral furrow formation and germband extension. We are imaging this medial pool at high temporal resolution to determine if its dynamics correlate with cell alignment.

174B

A novel concept of local homeostasis for the long-term maintenance of neuronal axons. Andreas Prokop[1], Yue Qu[1], Ines Hahn[1], Meredith Lees[1], Jill Parkin[1], Zhen Zang[2], Pakorn Tony Kanchanawong[2]. 1) Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom; 2) MBI, Singapore.

Axons are the slender processes of neurons which electrically wire the nervous system, and these delicate structures need to be maintained for decades. They are key lesion sites in trauma, neurodegenerative diseases and ageing. Parallel bundles of microtubules (MTs) form the structural backbones and life-sustaining transport highways of axons. Therefore, the formation and maintenance of ordered MT bundles is a key factor of axon development and longevity. However, the relevant underlying mechanisms are not known. Using systematic combinatorial genetics of numerous actin and MT regulators in Drosophila, we developed the novel concept of "local axon homeostasis" which proposes that different mechanisms of MT regulation act jointly all along axons to maintain organised MT bundles. We previously reported one mechanism, mediated by the actin-MT linker Shot, which ensures that MTs are laid into parallel bundles. Here, we report two further contributing mechanisms. First, a cortical MT collapse factor provides a novel check point mechanism which can eliminate MTs that leave the bundled organisation and go off track, and we will present our functional analyses. Secondly, we have discovered novel roles of cortical F-actin in axons. Rings of bundled, short, adducin-capped actin filaments, spaced by spectrin into a periodic pattern with 180 nm intervals, were recently described in mouse axons. With super-resolution microscopy we find similar repetitive actin structures in fly axons. Using pharmacological and genetic manipulations, we developed differential means to affect axonal actin, and our studies suggest that it plays prominent roles in axon growth promotion as well as MT and axon maintenance. In conclusion, our local homeostasis concept provides a new framework for studying the processes of axon development, ageing, degeneration and regeneration. -- Supported by the BBSRC, STFC, YQ's parents, Faculty of Life Sci.

175C

Key features of the axon initial segment are present in Drosophila neurons. Melissa M. Rolls[1], Michelle M. Nguyen[1], Daniel J. Goetschius[1], Timothy J. Jegla[2]. 1) Biochemistry and Molecular Biology, Penn State, University Park, PA; 2) Biology, Penn State, University Park, PA.

In mammalian neurons, the axon initial segment (AIS) is organized by ankyrin G. As ankyrin G is vertebrate-specific, the AIS is also thought to be found only in vertebrate neurons. However, there are recent reports that some channels and cytoskeletal proteins localize to segments of the proximal axon in both Drosophila and C. elegans neurons, and it has been known for many years that a spike initiation zone exists some distance from the cell body in axons of a variety of invertebrate neurons. We now demonstrate that multipolar Drosophila neurons have a diffusion barrier in the proximal axon. Moreover, as in mammals, this diffusion barrier relies on a neuronal ankyrin and a cell surface adhesion molecule. When either ankyrin-2 or neuroglian was reduced by RNAi, diffusion in the plasma membrane at the proximal axon increased. In addition, we show that it is specifically the long isoforms of ankyrin-2 that are required for this diffusion barrier. Previous channel localization studies in model invertebrates have been performed primarily on

unipolar neurons, making the comparison to mammalian multipolar neurons difficult. We therefore tested whether voltage-gated channels could be targeted to the region of the proximal axon where the diffusion barrier was found. We found tagged voltage-gated potassium and calcium channels concentrated in the proximal axon. Thus several key features of the AIS are found in Drosophila neurons: an ankyrin-dependent diffusion barrier and the ability to concentrate channels. We conclude that the major elements of the AIS were present in the common ancestor of ecdysozoans and chordates.

176A

Mutations in *Drosophila* crinkled/Myosin VIIA disrupt denticle morphogenesis. Jennifer Sallee[1,2], Janice Crawford[2], Vinay Singh[2], Daniel Kiehart[2]. 1) Biology, North Central College, Naperville, IL; 2) Department of Biology, Duke University, Durham, NC.

Actin filament crosslinking, bundling and molecular motor proteins are necessary for the assembly of epithelial projections such as microvilli, stereocilia, hairs, and bristles. Mutations in such proteins cause defects in the shape, structure, and function of these actin protrusions. One protein, Myosin VIIA, is an actin-based motor protein conserved throughout phylogeny. In *Drosophila melanogaster,* severe mutations in *crinkled* (*ck*) are "semi-lethal" with only a very small percentage of flies surviving to adulthood. Such survivors show morphological defects related to actin bundling in hairs and bristles and are deaf. To better understand *ck*/MyoVIIA's function in bundled-actin structures, we used dominant female sterile approaches to analyze the loss of maternal and zygotic *ck/*MyoVIIA in the morphogenesis of denticles, small actin-based projections on the ventral epidermis of *Drosophila* embryos. Maternal/zygotic *ck/*MyoVIIA mutants displayed severe defects in denticle morphology – actin filaments initiated in the correct location, but failed to elongate and bundle to form normal projections. Using deletion mutant constructs, we demonstrated that the motor domain and both of the C-terminal MyTH4 and FERM domains are necessary for proper denticle formation. Furthermore, we showed that *ck*/MyoVIIA interacts genetically with *dusky-like (dyl)*, a member of the ZPD family of proteins that links the extracellular matrix to the plasma membrane. Loss of one protein does not alter the localization of the other; however, loss of the two proteins together dramatically perturbs denticle shape. Our data indicate that *ck*/MyoVIIA plays a key role in arranging actin filament bundles into biologically functional units, which drive proper shape of cellular projections. .

177B

Actin regulators are important for regeneration in *Drosophila melanogaster* wing imaginal discs. Mabel Seto, Amanda R. Brock, Rachel K. Smith-Bolton. Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

All organisms require a mechanism of wound healing and/or tissue regeneration to survive in the wild. Tissue regeneration is a complicated process that requires the recognition of damaged tissue and the proliferation and repatterning of surrounding tissues. Previous studies in *Drosophila melanogaster* have shown that the actin cytoskeleton is involved in wound healing and tissue regeneration. Therefore, we screened for mutants of actin regulators that have an enhanced or diminished regeneration phenotype. We used a system of genetically induced ablation in the larval *Drosophila* imaginal wing disc, in which apoptosis is induced in the wing pouch in a spatially and temporally controlled manner. After ablation, the wing disc regenerates and the larvae undergo metamorphosis, allowing us to score the quality of regeneration based on the adult wing. The wings are screened semi-quantitatively and we have found mutations in actin regulators with both enhanced and reduced regenerative phenotypes. We present the results of our screen and an initial characterization of *Pnut* and *Btk29a,* which cause improved and impaired regeneration, respectively. This research will help us understand the role of the actin cytoskeleton in tissue regeneration and wound healing and how it is regulated during those processes.

178C

An optogenetic approach to assess tissue mechanics in epithelial wound detection. Erica Shannon[1], Monica Lacy[2], M. Shane Hutson[2], Andrea Page-McCaw[1]. 1) Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Physics, Vanderbilt University Collage of Arts and Sciences, Nashville, TN.

For an epithelial wound to heal, multiple cells surrounding the wound participate in a highly coordinated wound response. Even cells that are many cell lengths away from the wound site are capable of detecting and participating in the wound response. It has been hypothesized that mechanotransduction plays a role in wound detection. In this context, mechanotransduction describes how changes in tissue tension lead to the activation of signal transduction cascades. The cytoskeleton has extensive crosslinks and interactions with cell-cell junctions, making it well suited to transmit and transduce mechanical signals at wound sites. Actin, microtubules, and cell-cell junctions have all been implicated in mechanotransduction. We hypothesize that normal cytoskeletal structures are necessary for mechanotransduction that allows wound detection.

Because the cytoskeleton is critical for many cellular events, genetic analysis of its role in wound healing requires high resolution of spatial and temporal control of gene function. To achieve this level of spatiotemporal control, we are building a light inducible genetic circuit in *Drosophila* called GAVPO-FlpOn. In this optogenetic circuit, a light inducible Gal4-based construct (GAVPO, designed by Wang*et. al.* in 2012) uses a light-oxygen-voltage (LOV) domain to form transcriptionally active dimers when exposed to blue light. GAVPO activity drives the expression of Flippase, a DNA recombinase, which then induces the permanent expression of a Gene of Interest (GOI). We will use targeted laser irradiation to activate GAVPO-FlpOn in specific subgroups of cells within an epithelium to locally regulate cytoskeletal dynamics and assess the role of cytoskeletal mechanotransduction in the response to a wound.

179A

Polarized cytoskeletal organization in the *Drosophila* embryo. Alison Spencer[1], Shinya Yamamoto[2], Vafa Bayat[2], Manish Jaiswal[2], Nele Haelterman[2], Hugo J. Bellen[2], Jennifer A. Zallen[3]. 1) Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY; 2) HHMI and Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 3) HHMI and Developmental Biology Program, Sloan Kettering Institute, New York, NY.

Establishing structural organization, both within individual cells and throughout multicellular populations, is critical for proper tissue function. The epidermis of the late *Drosophila* embryo generates a series of highly organized actin structures called denticles whose reproducible banding patterns across the width of the animal have long been used as a readout of tissue patterning. Denticle cells also display a remarkable degree of subcellular organization: each cell generates actin structures that are simultaneously apically localized and planar polarized along the short axis of the cell, as well as microtubules that are oriented along the long axis. However, the mechanisms that regulate the subcellular organization of the actin and microtubule cytoskeleton in the denticle cells are not well understood. We have generated computational tools to quantify the spatial and temporal patterns of dynamic actin organization in denticle cells, and have performed a forward genetic screen on the X-chromosome to identify mutants that fail to establish this precise cytoskeletal pattern. We are currently characterizing cytoskeletal organization and the shape, polarity, and behavior of denticle forming cells in mutant embryos, and are working to identify the genes responsible for the mutant phenotypes. In addition, we are using live imaging approaches to elucidate the mechanisms by which these intracellular patterns of actin and microtubule organization are generated during development. .

180B

Apico-basal forces exerted by apoptotic cells drive epithelium folding. Bruno Monier[1], Melanie Gettings[1], Guillaume Gay[2], Thomas Mangeat[1], Sonia Schott[1], Ana Guarner[3], **Magali Suzanne**[1]. 1) LBCMCP, Toulouse University /CNRS, Toulouse, France; 2) DamCB company, Marseille, France; 3) Centro de Biología Molecular Severo Ochoa (C.S.I.C.-U.A.M.), Universidad Autónoma de Madrid, , Madrid, Spain.

Epithelium folding is a basic morphogenetic event essential to transform simple 2D epithelial sheets into 3D structures, in both vertebrates and invertebrates[1,2]. Folding has been shown to rely either on apical junction basal shifting or apical constriction [3-10]. The resulting cell shape changes depend on Myosin II redistribution which could be driven by mechanical signals[11]. Yet, the initial cellular mechanisms that trigger and coordinate cell remodelling remain largely unknown. Here, we unravel the active role of apoptotic cells in initiating morphogenesis, thus revealing a novel mechanism of epithelium folding. We show that, in a live developing tissue, apoptotic cells exert a transient pulling force upon the apical surface of the epithelium through a highly dynamic apico-basal Myosin II cable. The apoptotic cells then induce a non-autonomous increase in tissue tension together with cortical Myosin II apical stabilisation in the surrounding tissue, eventually resulting in epithelium folding. Together, our results, supported by a theoretical biophysical 3D model, identify an apoptotic Myosin II dependent signal as the initial signal leading to cell reorganisation and tissue folding. This work further reveals that, far from being passively eliminated as generally assumed (*e.g.* during digit individualisation[12]), apoptotic cells actively influence their surroundings and trigger tissue remodelling through regulation of tissue tension. .

181C

New cellular sites of prostaglandin regulated Fascin activity: the nucleus and nuclear periphery. Christopher Groen, **Tina Tootle**. Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

Here we present the novel finding that Fascin, a highly conserved actin bundling protein, localizes to new cellular sites. During *Drosophila* follicle development, in addition to being cytoplasmic, Fascin is also in the nuclei of the germline-derived nurse cells during stages 10B-12 (S10B-12) and at the nuclear periphery during stage 13 (S13). This localization is specific to Fascin, as other actin binding proteins, Villin and Profilin, do not exhibit the same subcellular distribution. Thus, Fascin localization to and functions at these new cellular sites are likely to be highly regulated. We find that loss of prostaglandin signaling causes increased nuclear Fascin during S10B, and a failure to relocalize to the nuclear periphery during S13. Loss of Fascin or increased nuclear Fascin results in opposing effects on nucleolar morphology, suggesting that prostaglandin signaling regulates nuclear Fascin levels to control nuclear organization. Given the numerous roles of Fascin in development and disease, including cancer, our novel finding that Fascin has functions within the nucleus sheds new light on the potential roles of Fascin in these contexts. .

182A

A composite scaffold provides structural integrity to myonuclei. Shuoshuo Wang, Talila Volk. Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Muscle nuclei are exposed to variable cytoplasmic strain produced by muscle contraction and release, but their morphology does not change. The mechanism responsible for maintaining myonuclear architecture is currently elusive. We uncover a unique myonuclear scaffold in Drosophila, exhibiting both elastic features, contributed by the stretching capacity of the KASH domain protein MSP-300, and rigidity provided by a perinuclear network of microtubules and their associated proteins the spectraplakin Shot and EB1. Together, they form a perinuclear flexible shield essential for protecting myonuclei from intrinsic or extrinsic forces. The loss of this scaffold resulted in significantly aberrant nuclear morphology and subsequent abnormal levels of essential nuclear factors.
Overall, we propose a novel mechanism for protecting myonuclear morphology and reveal its critical link to correct distribution of nuclear factors in differentiated muscle fibres. These findings may shed light on the underlying mechanism of various muscular dystrophies.

183B

Examining the regulation of Drosophila Rho kinase by phosphorylation. Y. Zhang, T. Jiang, T. Harris. University of Toronto, Toronto, Canada.

Actomyosin networks change cell shapes by generating contractile force. This process is activated by kinases such as Rho kinase (Rok). In mammalian cells, aPKC prevents over-activation of actomyosin by phosphorylating and restricting Rok localization. In Drosophila, whether aPKC inhibits actomyosin networks by phosphorylating Rok is unknown. We identified 11 putative aPKC phosphorylation sites in Drosophila Rok based on sequence comparisons with aPKC phosphorylation sites in mammalian Rok. Wild-type (Rok^{WT}) and non-phosphorylatable (Rok^{11A}) constructs of Rok were made, and their effects on epithelial tissue integrity and cellular actomyosin activity were evaluated. Both constructs induced embryonic lethality with overexpression, but Rok^{11A} produced a greater epithelial disruption. At cellularization both constructs induced a perpendicular expansion of furrow canal actomyosin networks, but Rok^{11A} produced a greater effect. In late embryos, Rok^{11A} but not Rok^{WT} formed abnormal foci with actin and phospho-myosin at the apical cortex of amnioserosa cells undergoing apical constriction. These elevated activities in Rok^{11A} were due to its kinase activity, as shown with additional mutation of the kinase domain. We hypothesized that the elevated Rok^{11A} activity could be due to altered protein localization and/or activation. In Drosophila embryos, both constructs accumulated at the apical circumference and apicomedial surface of epithelial cells similarly, suggesting the putative phosphorylation does not affect localization. Kinase-dead version of each construct also had similar localization, but both had lower cortical levels than the active constructs, indicating a kinase activity-dependent feedback mechanism.
Also, Rok^{11A} apicomedial networks persisted significantly longer than Rok^{WT} networks in constricting amnioserosa cells, as did individual Rok^{11A} puncta within the networks. Overall, we propose that one or more of the putative phosphorylation sites regulate Rok activity which in turn affects its localization through a positive feedback loop with actomyosin networks. We are currently testing aPKC phosphorylation of these putative sites to understand how upstream signals affect Rok activity.

184C

Continuous ROCK activity sustains apical constriction in a folding epithelium through the maintenance of centripetal tension. Jonathan Coravos, Adam Martin. Biology, Massachusetts Institute of Technology, Cambridge, MA.

Apical constriction transforms epithelial cells from a columnar to a wedge or cone shape, which can result in bending and folding of epithelial sheets during organogenesis. Apical constriction in several contexts, including *Drosophila* gastrulation, occurs through a progression from pulsatile to sustained contraction, suggesting that apical constriction reflects a dynamically established contractile cell state. This state, termed radial cell polarity (RCP), is characterized by a medial focus of Rho-associated coiled-coil kinase (ROCK) and a radiating actomyosin meshwork. Testing the model in which polarized ROCK is required to sustain contraction is difficult because genetic inactivation of ROCK prevents the early pulsatile phase of contraction. Thus, it is unclear whether ROCK is required for apical constriction through initial myosin activation, or sustaining apical constriction through constant myosin activation. To establish the temporal requirement of ROCK, we developed a simultaneous injection and live confocal microscopy technique to rapidly inhibit ROCK at a precise moment during tissue invagination. We find that ROCK activity is continuously required to sustain a contractile state in which actin, myosin, and ROCK are concentrated near the center of the apical surface and adherens junctions are present at the apical margin. Acute ROCK inhibition results in the rapid loss of myosin from the apical surface ($t_{1/2}$ = 15 seconds), suggesting that continued ROCK activity is required to maintain activated myosin, presumably by preventing dephosphorylation by myosin phosphatase.

185A

Tuning of a Par-1-Par-3-centrosome pathway to couple epithelial cell polarity and adhesion. Tao Jiang, Andrew McKinley, **Tony Harris**. Cell & Systems Biology, University of Toronto, Toronto, ON, Canada.

Epithelial plasma membranes contain apical and basolateral domains separated by circumferential cell-cell junctions. How junctions acquire even, ring-like distributions while associating with polarity networks that restrict molecules to single domains remains unclear. In *Drosophila*, aPKC regulates this coordination. Through quantitative imaging coupled with loss-of-function and suppression experiments, we have found that aPKC does so by inhibiting a positive feedback loop between the junctional organizer Bazooka (Baz)/Par-3 and centrosomes. Without aPKC, Baz and centrosomes lose their isotropic distributions and recruit each other to single plasma membrane foci through a positive feedback loop. Surprisingly, Par-1, a basolateral kinase known to phosphorylate Baz and inhibit its basolateral localization, is both necessary and sufficient for driving the Baz-centrosomes positive feedback loop. The ability of Par-1 to both locally promote and globally inhibit Baz complex assembly suggests a simple circuit for "Turing"-type polarization. We have evidence that Par-1 phosphorylation primes Baz for centrosome association, and are testing whether Baz de-phosphorylation is also needed since this phosphorylation has been shown to inhibit Baz clustering. Our data shows that aPKC normally restrains this polarization pathway by inhibiting Par-1, thereby allowing Baz and junctions to distribute more evenly around the apical circumference for the adhesion of epithelial cells.

186B

hosphorylation of DE-Cadherin Controls Adherens Junction Dynamics During Apical-Basal Polarization. Yang Hong, Yi-Jiun Chen, Juan Huang. Dept Cell Biol & Physiology, Univ Pittsburgh Med Sch, Pittsburgh, PA.

Apical-basal polarization in epitheila divides the cell into distinct apical and basal domains demarcated by cellular junctions such as tight junctions (TJ), adherens junctions (AJ), and septate junctions (SJ). To date, it remains unclear how junctional complexes may be dynamically regulated during apical-basal polarization. Using fluorescence recovery after photobleaching (FRAP) assays based on

genetically validated AJ markers in Drosophila, we discovered that DE-Cad and b-Catenin, two key components of AJ complex, had much faster biosynthetic turn-overs in polarizing epithelial cells than in polarized epithelial cells. To investigate whether such differential regulation of AJ dynamics is based on modulating the DE-Cad/b-Catenin interaction via DE-Cad phosphorylation, we have generated an extensive array of DE-Cad knock-in mutants that systematically mutate the conserved phosphorylatable Ser residues on the intracellular tail. These novel DE-Cad phosphor-mutants revealed that the conserved Ser residuals are quantitatively rather than qualitatively required for DE-Cad function and its binding to b-Catenin. FRAP assays confirmed that the different phosphorylation position of DE-Cad controls its biosynthetic turn-over separately. Interestingly, the lethality of non-phosphorylatable DE-Cad mutant can be fully rescued by fusion of a-Catenin (a-Cat) to the C-terminus of DE-Cad. Fusion of a-Catenin drastically decreases the biosynthetic turnover of non-phosphorylatable DE-Cad, indicating that, when covalently bound to DE-Cad, a-Catenin can substitute the b-Catenin function in forming functional and much stable AJ complexes. Overall, our data suggest that phosphorylation of DE-Cad serves as a key mechanism regulating the AJ dynamics in vivo during apical-basal polarization.

187C

The role of Moesin in specialized membrane domain formation during early *Drosophila* embryogenesis. Danielle Howell[1], Michael Ludwig[2], Xiao Sun[3], Richard Fehon[4]. 1) Development, Regenerative and Stem Cells Biology, University of Chicago, Chicago, IL; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 3) Peking University, Beijing, China; 4) Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Specialized membrane domains are essential for epithelial function. One such domain, the sub-apical region, is of special interest because it positions and stabilizes the adherens junctions. Adherens junctions form a ring in epithelial cells, and it is this structure, along with the underlying actomyosin network, that connects neighboring cells and supports the epithelial sheet. We are interested in both how this region forms correctly and how subapical region stability is maintained, particularly in relation to Moesin, a FERM domain protein essential for proper epithelial integrity. Loss of Moe causes delamination, cell rounding, apoptosis, and ectopic f-actin accumulation in established epithelia. The exact mechanism by which Moesin affects these cellular processes is unknown, though Moesin is well established as a membrane-cytoskeleton cross-linker, and is known to function in regulation of RhoA activity.
We are focusing our investigation on the role of Moesin in early *Drosophila* embryogenesis. Specifically, we are studying the process of cellularization, during which embryos undergo rapid membrane ingression to build cells around thousands of nuclei simultaneously. The formation of the cells *de novo* creates an ideal environment to study Moesin's role in membrane domain formation and organization. We are utilizing recently developed tools to acutely deplete Moesin prior to cellularization, allowing us to assess its role in junction and polarity formation. .

188A

Intracellular mechanisms involving post-translational modifications of the PIP5K Skittles regulate PI(4,5)P2 associated cell polarity. Julie Jouette, Antoine Guichet, Sandra Claret. Institut Jacques Monod, Paris, France.

The control of apical-basal polarity in epithelial layers is a fundamental process. A key feature of polarized cells is their ability to maintain an asymmetric distribution of specific molecular complexes, including the phosphoinositide PI(4,5)P2. Using the *Drosophila* egg chamber as model system, we have recently showed that in the follicular epithelium, PI(4,5)P2 is essentially regulated by the PIP5K Skittles (SKTL) and is crucial to maintain apical-basal polarity. We have also found that SKTL, by controlling PI(4,5)P2 polarity, regulates the apical targeting of PAR-3 to the plasma membrane and thus the size of the apical domain (Claret et al., 2014). We had previously shown that in the oocyte SKTL regulates PAR polarity proteins and the maintenance of specific cortical domains along the antero-posterior axis (Gervais et al., 2008).
Surprisingly, in epithelial cells, only a discrete fraction of SKTL exhibits a polarized distribution similar to its product, PI(4,5)P2. In accordance with this, overexpressed SKTL frequently fails to reach the apical domain. This suggests that a robust process tightly controls the apical accumulation of SKTL. To investigate this process, we are exploring SKTL regulation through post-translational modifications and testing their importance for cell polarity associated SKTL function. We found that SKTL undergoes multiple modifications including phosphorylations and a palmitoylation. Furthermore, by combining mass spectrometry and co-immunoprecipitation approaches we identified several kinases and one phosphatase that are potentially involved in SKTL post-translational modifications. Finally by searching for palmitoyl-modifying enzymes, we identified one gene encoding a palmitoyltransferase, which upon knock-down by RNAi, affects cell polarity in the oocyte. In that case, SKTL, normally located at the plasma membrane, is delocalized in large ring structures in the cytoplasm.
PI(4,5)P2 is involved in the regulation of a number of cellular activities including vesicular trafficking, cytoskeleton organisation and cell motility. This work could also enlighten about the spatiotemporal regulation of this phosphoinositide by the PI4P5 kinases.

189B

Dachsous, Fat and actin modulators contribute to planar cell polarity in the Drosophila ventral epidermis. Gregory Osborn, Dan Ly, Kynan Lawlor, Stephen DiNardo. Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

Morphogenesis is orchestrated by the cooperation of many cells: for proper execution, cells must respond to directional information and transmit these cues to neighboring cells. To accomplish this, epithelial cells tend to establish polarity within the plane of the epithelium, in addition to apicobasal polarity. Such planar cell polarization (PCP) allows cells to define their position within a field, thereby allowing cells to coordinately produce asymmetric responses essential for tissue function. The molecules that control planar cell polarity have been identified and well studied; polarizing systems such as the conserved Frizzled (Fz) and the Dachsous/Fat (Ds/Fat)

systems allow cellular neighbors to establish and maintain polarity. Less understood is how these systems integrate with downstream effectors to choreograph asymmetric responses. Here we study the *Drosophila* ventral epidermis, an epithelium in which cells produce a stereotyped template for the production of denticles, critical for larval motility. This template is comprised of actin-based protrusions (ABPs), which are asymmetrically polarized within these cells. The Ds/Fat system is essential for the proper localization of ABPs; the current model is that Ds signals through the Fat receptor to impose polarity. We are performing functional domain analysis of Fat, in order to determine critical motifs of this molecule and to predict downstream effectors of the Ds/Fat system. In addition, through live imaging and genetic techniques, we have begun analysis of candidate cytoskeletal molecules (actin modulators, myosin II) to identify the machinery that engages in the construction and placement ABPs at the posterior edge of these cells. By working from receptor to cytoskeleton, we hope to understand how molecular polarity determinants integrate with structural effectors to functionally polarize tissue, a process critical in both development and disease.

190C

Exploring the mechanism of Prickle/Spiny-legs isoforms' control of microtubule polarity and symmetry breaking in planar cell polarity. Katherine Sharp[1,2], Jessica Olofsson[1,3], Jeffrey Axelrod[1]. 1) Department of Pathology, Stanford University School of Medicine, Stanford, CA; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 3) Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.

Planar cell polarity (PCP) is the alignment of cells within the plane of the epithelium. For robust PCP signaling to occur, all the cells in a tissue must break symmetry in the same direction with respect to the axis of the tissue such that certain proteins accumulate on one side of each cell and other proteins accumulate on the opposite side. We and others have previously shown that, in the developing wing and abdomen of the fly, this coordinating symmetry breaking is accomplished via directed trafficking of vesicles containing Fz and Dsh along an apical network of microtubules (MTs). We further showed that alternate Prickle protein isoforms bias the polarity of these MTs in opposite directions thereby controlling the directional bias of vesicle trafficking such that expression of one isoform versus the other determines the direction of symmetry breaking within a given tissue. PCP signaling is required in the fly wing for the proper alignment of the small trichomes (hairs) that cover the adult cuticle of this tissue. We have observed that while Prickle isoform expression can determine the direction of hair growth across the entire wing, MT polarity is biased only in the proximal part of the wing and that altering Prickle isoform expression has no effect on MT polarity in the distal region of the wing. We are therefore exploring how symmetry breaking is regulated in this distal region. Additionally, we are conducting a candidate-based RNAi screen to identify proteins that help Prickle isoforms control MT polarity. Finally, we are making deletion constructs of the Prickle isoforms that we will use to explore the functional importance of different portions of these proteins. .

191A

Planar polarity and oriented cell division generate an epithelial square grid in the *Drosophila* embryo. Masako Tamada[1,2*], Jennifer Zallen[1,2]. 1) Howard Hughes Medical Institute; 2) Dev Biol, Sloan Kettering Institution, New York, NY.

Tissue morphogenesis is often characterized by increasing cellular disorder, but differentiated tissues ultimately display highly spatially ordered patterns. Although tissue patterning genes have been identified in many tissues, the mechanisms by which the expression of these genes control cellular behaviors in time and space to arrange disordered cells into distinct patterns are not well understood. We found that the *Drosophila* pharynx epithelium displays a unique structure consisting of four rows of square-shaped cells tiled in a highly regular grid. Square grid cells display a striking planar polarized localization of contractile and adhesive proteins, and they become organized through three sequential processes. In Phase I, cells intercalate and directionally rearrange to produce two rows of rectangular-shaped cells flanking the midline. In Phase II, oriented and synchronized mitoses convert these into four parallel rows of cells that maintain a square shape. In Phase III, the daughter cells elongate 1.6-fold along the apical-basal axis to form a compact square grid. We found that the square cell grid does not form in embryos lacking the basic leucine zipper transcription factor Cap 'n' collar/NFE2 (Cnc) is necessary for square grid formation. In addition, Cnc misexpression throughout the embryo is sufficient to reorient the planar polarity of cells and induce ectopic square grid formation along the entire ventral midline. The conserved Pins/LGN protein accumulates asymmetrically within dividing cells and orients the axis of planar cell divisions dorsoventrally, and that is critical to square grid formation. Midline cells are also necessary for square grid formation and Pins is selectively recruited to contacts between midline cells and Cnc-positive square grid-forming cells. These results demonstrate that localized patterns of gene expression direct ordered cellular patterns in tissue organization through the spatiotemporal regulation of oriented cell divisions.

192B

Parkinson's disease gene *Vps35* regulates autophagy in *Drosophila* neurons. Y.-J. Ho, R. Linhart, D. Kaing, E. Hou, D. Sohal, R. Fedrizzi, A. Tsang, D. Fong, R. Eismati, J. Rosales, A. Dawson, J. Ly, C. Kim, K. Venderova. Physiology and Pharmacology, University of the Pacific, Stockton, CA.

VPS35 is a confirmed causative gene of Parkinson's disease. The gene codes for the core component of the retromer complex known to be responsible for sorting and trafficking of select cargo proteins from endosomes to the trans-Golgi network or plasma membrane. However, how *VPS35* contributes to Parkinson's disease pathology is unknown. We demonstrate that overexpressing its *Drosophila*homologue, *Vps35*, greatly exacerbated the eye phenotype of flies overexpressing Atg1 – a kinase necessary for autophagy initiation. Conversely, knocking-down expression of Vps35 rescued the Atg1 phenotype. These data suggest that Vps35 positively regulates autophagy. Next, we analyzed this interaction in dopaminergic neurons that control movement. We previously demonstrated that reducing expression of Vps35 in *Drosophila* dopaminergic neurons causes locomotor deficits. While

overexpressing *Atg1* alone had no effect on locomotor activity, it failed to rescue phenotype of the *Vps35* knock-down flies. Altogether, our data suggest that retromer is acting downstream of Atg1 to positively regulate autophagy.
This research is supported by NIH (1R03NS088913-01 to KV).

193C

A rapid, membrane-dependent pathway directs furrow formation through RalA in the early *Drosophila* embryo. R. Holly, L. Mavor, Z. Zuo, T. Blankenship. University of Denver, Denver, CO.

Plasma membrane furrow formation is a critical part of cell division and cytokinesis. Furrow formation in early syncytial *Drosophila* embryos is exceptionally rapid, with furrows forming in as little as 3.5 minutes. Here, we use 4D imaging to identify furrow formation, stabilization, and regression periods, and identify a rapid, membrane-dependent pathway that is essential for plasma membrane furrow formation *in vivo*. While Myosin II function is canonically thought to provide the ingression force for cytokinetic furrows, the role of membrane trafficking pathways in guiding furrow formation is less clear. We demonstrate that a membrane trafficking pathway centered on the Ras-like protein A, RalA, is required for fast furrow ingression in the early fly embryo. We show that *RalA* function is absolutely required for furrow formation and initiation. In the absence of RalA function, chromosomal segregation is aberrant and polyploid nuclei are observed. RalA localizes to the syncytial furrows, and mediates the movement of exocytic vesicles to incipient furrows. Sec5, which is an exocyst complex subunit and localizes to ingressing furrows in wild type embryos, becomes punctate and loses its cortical association in the absence of RalA function. Rab8 is also unable to traffic to the plasma membrane and accumulates aberrantly in the cytoplasm in RalA disrupted embryos. Interestingly, RalA germline clone embryos demonstrate an absence of F-actin polymerization at the base of the furrow canal, suggesting that membrane addition is required for, and may precede, cytoskeletal remodeling during furrow formation. These studies identify a pathway that stretches from Rab8 to RalA and the exocyst complex that mediates rapid furrow formation in the early *Drosophila* embryo. These results further suggest that a brick-and-mortar model of membrane addition may contribute to traditional cytokinetic furrow ingression.

194A

Rab8 directs tubulation and furrow ingression during epithelial formation in *Drosophila melanogaster*. L. M. Mavor, Z. Zuo, J. T. Blankenship. Biological Sciences, University of Denver, Denver, CO.

One of the most fundamental cell shape changes that cells perform is the ingression of a plasma membrane furrow. The *Drosophila* embryo undergoes several cycles of rapid furrow ingression during early development that culminates in the formation of an epithelial sheet. Previous studies have demonstrated the requirement for intracellular trafficking pathways in furrow ingression; however, the pathways that link compartmental behaviors with cortical furrow ingression events have remained unclear. Here, we show that Rab8 demonstrates striking dynamic behaviors *in vivo*, forming vesicular and tubular structures that precede the actomyosin network associated with furrow canals. After early furrow initiation, Rab8 transitions to a cortical location that coincides with known regions of directed plasma membrane addition. When Rab8 function is disrupted, furrow formation in the early embryo is completely abolished. We additionally demonstrate that Rab8 behaviors are dependent on actin filament formation for proper orientation, while microtubule networks are essential for Rab8 tubule formation but dispensable once a Rab8 cortical array has formed. Active, GTP-locked Rab8 is primarily associated with dynamic membrane compartments and the cortical array, while GDP-locked Rab8 forms large cytoplasmic aggregates. Additionally, RNAi knockdown of Rab11 and Sec5 demonstrates that Rab8 functions in a pathway that requires both the recycling endosome and exocyst in order to localize and behave dynamically with the cortex of ingressing furrows. *In vivo* imaging alongside functional knockdown of Rab8 demonstrates that Rab8 functions as a critical mediator of furrow ingression. These studies suggest that active, membrane-bound Rab8 populations prefigure and initiate furrow ingression, and direct tubulation and trafficking behaviors in the *Drosophila* embryo.

195B

Dynamin-mediated endocytosis promotes cell intercalation during epithelial tube morphogenesis in the *Drosophila* ovary. Nathaniel Peters, Kamsi Odinammadu, Celeste Berg. Department of Genome Sciences, University of Washington, Seattle, WA.

Epithelial tube morphogenesis (tubulogenesis) is critical for organ development and requires the integration of diverse cellular processes governing shape and movement. In the fly ovary, epithelial follicle cells encasing developing oocytes execute a robust tubulogenic program to create the eggshell's dorsal respiratory appendages (DAs). Since DA-tubulogenesis occurs without cell division or death, it provides a useful and tractable model for understanding the contributions of cell shape, movement, and organization to tubulogenesis. We previously identified the GTPase Dynamin as a downstream effector of Tramtrack69, a transcription factor that promotes DA-tube expansion (Peters *et al.,* 2013). Here we explore the mechanistic action of Dynamin in DA-tubulogenesis by disrupting function with dominant negative Dynamin (DN-Dyn). When we express DN-Dyn throughout the follicle cells, eggs exhibit short, wide, and flat DAs with irregular edges, suggesting defects in cell intercalation, adhesion, and/or migration. Expression of DN-Rab5 or AP-2µ RNAi produces similar defects, supporting the hypothesis that Dynamin's function in DA-tubulogenesis is to promote endocytosis. Interestingly, we observe distinct DA defects if we express DN-Dyn only in DA-tube cells, or only in stretch follicle cells over which the DA-tube cells migrate. Analysis of DA-tube cell arrangement in fixed tissue and live imaging of cultured DN-Dyn egg chambers indicate that DN-Dyn DA-tube cells fail to intercalate and extend the DA tubes. These results suggest that Dynamin-mediated recycling of cellular adhesions is important for interaclation. Since we observe high Dynamin protein levels at both apical and basal membranes, we analyzed apical E-Cadherin and basal B-Integrin in DN-Dyn DA-tube cells; we found stabilization and/or altered

behavior of both proteins. To test whether excess adhesion proteins are the cause or the effect of the DN-Dyn phenotype, we are over-expressing these proteins in a wild-type background. Preliminary data suggest that over-expressing E-Cadherin alone produces eggs with short, widened, DAs. We propose that endocytic recycling of cellular adhesions in DA-tube cells promotes intercalation and drives DA-tube expansion.

196C

The ArfGAP Asap is required for plasma membrane furrow formation in the syncytial *Drosophila* embryo. Francisco Rodrigues, Tony Harris. Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada.

In the *Drosophila* syncytial embryo, cortical nuclei undergo multiple rounds of division that are associated with the dynamic ingression and regression of plasma membrane (PM) furrows that keep individual mitotic machinery separate. Different endocytic pathways regulate this process in distinct ways. At the apical cell surface, transcytosis fuels the ingression of invaginating membranes. Additionally, at PM furrow tips, the ArfGEF (guanine exchange factor) Steppke restrains excessive membrane growth by promoting local endocytosis via the activation of Arf signaling. To investigate the role of an ArfGAP (GTPase activating protein), we pursued Asap. A GFP-tagged Asap construct was enriched at the apical cell surface and the tips of invaginating PM, consistent with a role in endocytic trafficking. To determine its role at the PM, we depleted endogenous Asap using shRNAs. Embryos depleted of Asap often displayed a complete absence of furrows separating individual nuclei. We observed more embryos with this phenotype with *asap*^{shRNA} plus *steppke*^{shRNA}, suggesting that Asap and Steppke act cooperatively rather than antagonistically to produce PM furrows. To further probe how Asap and Steppke associate, we co-expressed both proteins. Surprisingly, co-expression led to an increase in cortical levels of both Asap and Steppke compared to the expression of either alone. By using GEF and GAP defective constructs, we showed that this reciprocal recruitment was dependent on their catalytic activities. Our lab has shown that overexpression of Steppke alone leads to abnormal furrow loss. When co-expressed, Asap GAP activity was able to suppress Steppke's effects on furrow tips, indicating an antagonistic relationship in this case. We propose a model in which Asap promotes furrow formation in two distinct ways: at the apical surface, Asap might form a kinetic scaffold required for the local supply of ArfGDP for Steppke and other ArfGEFs to activate and thus promote endocytosis; and at invaginating PM furrow tips, Asap can inactivate Arf signaling to prevent excessive endocytosis. Overall, these data suggest that a single type of ArfGAP both promotes and hinders an ArfGEF based on local conditions at different sites in the cell.

197A

Sequential trafficking events target White transporter to pigment granules. DS Chen[1], J Xiong[1,3], YN Rui[1], A Tito[1,3], Z Chen[1], Z Xu[1], L Ye[1], G Jiang[4], **S Zhang**[1,2,3]. 1) Inst Mol Med; 2) Dept Neurobio Anatomy, UTHSC-H; 3) Programs in HMG and Neuroscience, University of Texas Graduate School of Biomedical Sciences, Houston, TX; 4) Shandong Univ, School of Medicine, China.

white, the eye color gene associated with the first reported *Drosophila* mutant, is also the most widely used marker in fly studies. Despite this long history and its importance, few systematic studies on its expression and regulation have been reported. *white* encodes an ABCG family half transporter proposed to form heterodimers with Brown and Scarlet of the same transporter family to package red and brown pigments, respectively, into pigment granules (PG), the only known lysosomal-related organelle (LRO) in *Drosophila*. Among the large number of existing eye color mutants, the "granule group" genes are known to affect eye pigmentation by regulating the biogenesis of PG, potentially through controlling the subcellular trafficking of membrane proteins destined for this organelle. However, whether and how White is regulated through this process is not known.

Using specific anti-White antibodies and genome-tagged White transgenes, we show that White is glycosylated and its expression starts from early during embryogenesis well into adulthood. At subcellular level, White is specifically localized on the limiting membrane of the PG, which can be further divided into two distinctive subpopulations based on their unique morphologies which we propose are defined by Brown and Starlet. Moreover, systematic examination of its distribution in a series of eye color mutants reveals distinctive abnormal subcellular distribution of White in different granule group mutants, supporting a sequential trafficking events mediated by discrete protein complexes for faithful targeting of White to PG. Finally, our study reveal an absolutely requirement for Brown and Starlet transporters in the proper expression of White protein. These findings thus lay foundation for future detailed dissection of White trafficking, LRO biogenesis and LRO-related diseases using *Drosophila*..

198B

Polarization of the epithelial layer is required for apical localization of the integrin heterodimer, αPS3βPS, to promote engulfment. Tracy Meehan[1], Sarah Kleinsorge[2], Allison Timmons[1], Jeffrey Taylor[1], Kimberly McCall[1]. 1) Department of Biology, Boston University, Boston, MA; 2) Graduate Program of Genetics and Genomics, Boston University Medical Center, Boston, MA.

Epithelial cells, such as mammalian retinal pigment epithelium (RPE) cells, are crucial non-professional phagocytes which engulf daily to clear corpses that would otherwise lead to conditions such as macular degeneration. One of the engulfment receptors present on the apical surface of RPE cells is integrins, which are more commonly basally localized. Interestingly, the follicle cells in the *Drosophila*ovary also engulf germline apoptotic debris via their apical side. We found that the integrin subunits αPS3/βPS increase apically on the follicle cells and are required for engulfment. Little is known about integrin localization and trafficking in engulfing cells. Surprisingly, we found that integrin heterodimer localization and function is largely determined by the α subunit. We also determined that proper polarity of follicle cells is essential for engulfment and integrin enrichment, suggesting that αPS3/βPS trafficking occurs in a polarized fashion. We screened through several candidate genes known to regulate integrin function in other contexts, and found that several of the genes required for integrin enrichment in the follicle cells have been previously shown to play a role in migration, but have no previously

reported role in non-professional phagocytosis. Migration machinery has been associated with engulfment in macrophages, but these results suggest that the utilization of migration genes may be a universal feature of engulfing cells. Our findings demonstrate a critical role for integrin function, trafficking, and competition between integrin subunits during engulfment. Moreover, as in mammals, we have found that the same α subunit is required by both professional and non-professional phagocytes.

199C

Polarized transport and selective retention localizes the signaling center for bristle cell elongation. T. Otani[1], K. Oshima[1], A. Kimpara[1], M. Takeda[1], U. Abdu[2], S. Hayashi[1]. 1) Lab Morphogenetic Signaling, RIKEN Ctr Dev Biol, Kobe, Japan; 2) Dept of Life Sciences, Ben-Gurion Univ, Beer-Sheva, Israel.

Accurate transport of proteins and vesicles is essential for cellular morphogenesis and physiology. We have been studying the dynamics of intracellular transport during Drosophila bristle cell elongation, and have previously reported that Rab11-positive recycling endosomes undergo a dynamic shuttling movement between the cell body and the distal tip (Otani et al., Dev Cell, 2011). Moreover, a protein kinase IKKε/IK2 localizes to the distal tip of the growing bristles, and acts as a signaling center that regulates recycling endosome shuttling. However, how IKKε can localize to the distal tip remains unknown. We now demonstrate that IKKε localizes to the distal tip by cytoplasmic dynein-dependent polarized transport. Spindle-F (Spn-F), an adaptor protein, is required for this polarized transport, and acts as a molecular linker between IKKε and cytoplasmic dynein. Notably, photobleaching experiments revealed that SpnF-GFP does not undergo bidirectional shuttling movement, but is stably localized at the distal tip. Thus, although Rab11 and Spn-F are both transported to the distal tip by cytoplasmic dynein, the transport dynamics are differentially regulated – Rab11 is transported back to the cell body, whereas Spn-F is selectively retained at the distal tip. We identify Javelin-like (Jvl), a coiled-coil protein as a key regulator of Spn-F retention. In *jvl* mutant bristles, IKKε and Spn-F can initially localize to the distal tip, but fail to be maintained. Intriguingly, in S2 cells, Jvl or Spn-F single-positive particles dynamically move in microtubule-dependent manner, whereas Jvl/Spn-F double-positive particles are immobile, indicating that Jvl can immobilize Spn-F. Taken together, these results suggest that polarized transport and selective retention regulates the distal tip localization of IKKε/Spn-F complex during bristle cell elongation.

200A

Novel smad protein dysmorphic regulates Drosophila tracheal tube size through luminal matrix maintenance. Rachana Radhamani Chandran, Lan Jiang. Department of Biological sciences, Oakland University, Rochester, MI.

Tubular organs are important structural components of metazoans. Proper tube dimensions are critical for the functioning of such organs. Defective tube dimensions can cause devastating human diseases such as polycystic kidney disease and fibrocystic breast disease. We identified a novel gene, *dysmorphic*, which is expressed mainly in multicellular branches. Phenotypic analysis of *dysmorphic* mutants displays elongated tracheal dorsal trunk (multicellular branch) with irregular diameter, suggesting that this gene controls tracheal dimensions. Proper localization of tracheal luminal proteins and septate junction components along with timely protein turn over facilitated by Rab mediated endocytosis are important factors that control tracheal tube size. No obvious defects in the distribution of septate junction components were observed in *dysmorphic* mutants. However, immuno histochemical analysis shows that luminal matrix components such as chitin, chitin modifying enzymes vermiform and serpentine, and chitin binding protein gasp exhibit suboptimal levels during later stages of tracheal development. The synthesis and secretion for these luminal proteins remain unaffected as indicated by the normal abundance of luminal proteins at the early stages. These results suggest that the maintenance of luminal components is defective in *dysmorphic* mutants. Interestingly, Obstructor-A, another luminal matrix component, which prevents the luminal matrix from premature degradation, failed to secrete to the lumen in *dysmorphic* mutants. To test the possibility that dysmorphic might be required for the assembly and secretion of Obstructor-A containing cargo, a protein pull down assay was performed. As expected, Obstructor-A was immuno-precipitated along with dysmorphic. In addition, MyoV and Rab11, two proteins involved in apical secretion, were also pulled down by dysmorphic. Protein interaction assay strongly suggests that dysmorphic may form a complex with the Obstructor-A containing cargo. Then, Rab11 along with motor protein MyoV may assist the apical secretion of Obstructor-A. Luminal Obstructor-A in turn maintains the rest of the luminal matrix components to control tube size. .

201B

Dosage-sensitive interactions between Kinesin-1 and transiently expressed Halo regulate lipid-droplet transport. Michael Welte, Gurpreet Arora, Susan Tran, Nicholas Rizzo. Dept Biol, RC Box 270211, Univ Rochester, Rochester, NY.

In early *Drosophila* embryos, lipid droplets exhibit coordinated bidirectional transport, powered by Kinesin-1 and cytoplasmic Dynein. Motor activity on droplets is temporally regulated, resulting in global shifts of the entire droplet population as embryogenesis proceeds. Halo is the key factor controlling this timing; its new expression upregulates Kinesin-1 activity on droplets and thus mediates the switch to net plus-end transport in Phase II (early cycle 14). Halo is rate limiting as Halo dosage reduction delays this switch while an increase in Halo dosage accelerates it. During Phase III (gastrulation), Halo mRNA and protein levels drop sharply and net motion is minus-end directed. We find that in embryos mutant for the lin-41/TRIM71 ortholog Wech, Halo mRNA and protein levels remain high and motion stays plus-end directed in Phase III; thus, abrogating Halo expression may be essential for the switch in net directionality. Previous work suggested three distinct models for how Halo acts molecularly: It might be a transcription factor, a phosphatase acting on the lipid-droplet protein LSD-2, or a motor cofactor. Halo's intracellular distribution is consistent with all three models: Halo is present in nuclei as well as in the cytoplasm, and a small fraction copurifies with lipid droplets. Providing Halo (from injected *halo* mRNA) is sufficient to affect droplet motion even when transcription is pharmacologically inhibited; thus, Halo does not control transport by regulating transcription. Epistasis analysis revealed that Halo modulates transport even in the absence of LSD-2; thus Halo can act independent of

LSD-2. Using immunoprecipitation, we find that Halo and Kinesin-1 exist in common protein complexes in the embryo cytoplasm. Kinesin-1 and Halo indeed function in the same pathway as absence of either leads to failure of net plus-end transport during Phase II. Reducing Halo dosage delays this net plus-end transport, but – surprisingly – when levels of Halo and Kinesin-1 are reduced simultaneously, transport kinetics reverts back to wild type. We show that the critical parameter is the Kinesin-1/Halo ratio and propose that Halo is a limiting cofactor whose binding to Kinesin-1 increases motor processivity.

202C

Content release from salivary-gland secretory vesicles is mediated by a dynamic acto-myosin network. Eyal Schejter, Tal Rousso, Ben-Zion Shilo. Dept Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

Secretion of viscous materials by exocrine cells requires the exertion of active forces, to facilitate content release from large secretory vesicles at late stages of exocytosis. A universal feature of such systems is formation of an actin coat around each vesicle, which is thought to mediate the forces necessary to complete the secretory process. Using live imaging of cultured *Drosophila* larval salivary glands, an established model for such secretory systems, we have followed the dynamics of actin coat formation around "glue"-filled vesicles, and vesicle content release. Larval salivary glands glands are well-suited for such studies, due to the exceptional imaging qualities provided by their unique size and architecture, and the ability to apply refined genetic manipulations at the level of the whole organism. Our analysis of glue vesicle exocytosis demonstrates that the Formin family protein Diaphanous (Dia) is critical for generation of the actin coat structure, and is recruited onto the secretory vesicle surface following its fusion with the plasma membrane. "Squeezing" of the actin-coated vesicle and content release require Myosin-II, which is recruited onto the fusing vesicle as a result of a signaling cascade involving Rho1 and Rho kinase (Rok). Myosin-II exhibits a unique organization that coincides with sites of contraction along the vesicle, and sheds light on the forces that are applied on the vesicle to facilitate efficient content release.

203A

Myosin activity at the periphery of the border cell cluster promotes its compact shape during migration between nurse cells. George Aranjuez[1,2], Pralay Majumder[1], Ashley Burtscher[1], Jocelyn McDonald[1,2]. 1) Molecular Genetics, Cleveland Clinic Foundation, Cleveland, OH; 2) Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH.

The coordinated movement of multiple cells, termed collective migration, features prominently in normal development, wound healing, and cancer metastasis. Migrating collectives organize and maintain group cohesion despite mechanical constraints imposed by the extracellular environment. However, the mechanisms underlying these processes are poorly understood. Migrating border cell clusters in the*Drosophila* ovary are an excellent model to study how groups of migrating cells interact with their environment. We and others have previously shown that non-muscle myosin II (myo-II) is required for border cell migration, detachment from the follicle epithelium and leading edge protrusion dynamics. Here, we demonstrate that myo-II is further required for the compact shape of the cluster during their migration between nurse cells. Activation of myo-II is achieved via phosphorylation of the regulatory light chain, Spaghetti Squash (Sqh). Loss of *sqh* or of the known myo-II kinase *Rho kinase* (*Rok*) resulted in elongated clusters, which were stretched along the migration path. Conversely, high myo-II activity led to overcompaction of the cluster. Both conditions were accompanied by a failure to complete migration. Furthermore, Rok and phosphorylated Sqh localized specifically to the cluster periphery in small foci or 'hotspots', suggesting that such localized myo-II activity is important for collective migration and cluster shape. Phosphomimetic Sqh rescued the migration and protrusion defects of *Rok* mutants but, surprisingly, not the elongated cluster phenotype. We favor the hypothesis that cluster shape requires pulses of myo-II activity at the periphery, which the phosphomimetic Sqh mutant cannot facilitate. Finally, we are currently performing live imaging to determine how wildtype or *sqh* mutant clusters respond to changes in nurse cell tension. Our model is that myo-II activity at the cluster periphery counteracts the mechanical constraints imposed by the nurse cells to maintain cluster shape and promote collective migration.

204B

***Tsp66E*, the KAI1 homologue, functions in the border cell migration and maintaining the adhesion of premature border cells in *Drosophila*. Haemin Jeong**, Seung Yeop Han, Minjung Lee, Soojin Lee, Myeong Cheol Shin, Young Jae Jeon, Kyoung Sang Cho. Department of biological sciences, Konkuk university, Seoul, South Korea.

KAI1/CD82, a member of tetraspanin, has been known for a tumor metastasis suppressor, and implicated in cell-cell adhesion and cell migration in various tumor cells. However, the functions of KAI1/CD82 in migration of normal cells are not known well. To understand it, we investigated the role of *Tsp66E, Drosophila* homologues of *KAI1/CD82,* in the border cell migration, which is a well-established model for cell migration *in vivo*. In this study, the border cell migration was accelerated by loss of *Tsp66E*, and delayed by *Tsp66E* overexpression. *Tsp66E*-deficiency increased the level of βPS integrin, which is an accelerator of cell migration, in ovaries compared to wild type. Moreover, the migration of *Tsp66E*-overexpressing border cells was further delayed by RNAi knockdown of*myospheroid*, a gene for βPS integrin. In addition, the *Tsp66E* deficiency enhanced the premature detachment phenotype of the border cells at stage 8 of *Drosophila* oogenesis in αPS2 integrin, *inflated*, mutant. Interestingly, these phenotypes became more pronounced by deficiency of another tetraspanin gene, *Tsp74F*. Taken together, our results suggest that Tsp66E negatively regulates the border cell migration in *Drosophila*, and that Tsp66E and Tsp74F cooperate with integrin in the maintaining adhesion of the premature border cells.

205C

LAMININS ARE REQUIRED FOR PROPER MIGRATION OF EMBRYONIC HAEMOCYTES IN *DROSOPHILA MELANOGASTER*. Besaid J Sánchez-Sánchez[1], J. M. Urbano[2], K. Comber[3], W. Wood[3], M. D. Martín-Bermudo[1]. 1) CABD, CSIC, Sevilla, Spain; 2) PDN Department, University of Cambridge, UK; 3) Department of Developmental Biology, University of Bristol, UK.

The extracellular matrix (ECM) covers the basal side of all epithelia and endothelia and surrounds muscles, peripheral nerves and other tissues, providing physical support. In addition, the ECM has been implicated in many processes such as cell differentiation, shape, adhesion, survival and migration. The main producers of ECM in the embryo are fat body and macrophages (haemocytes). Embryonic haemocytes not only constitute the first line of defence against infection but also help to sculpt organs and tissues by removing dead cells. Key to their function is their ability to migrate and disperse throughout the embryo. Yet, despite these important developmental functions, little is known about the molecular mechanisms underlying embryonic haemocyte migration in vivo.
Using *Drosophila melanogaster* as a model system, we have analysed the role of different ECM components, such as Laminins, Collagen IV and Perlecan, during embryonic haemocyte migration. Our results show that Laminins are the main ECM components supporting haemocyte migration. Among the two Laminin trimers present in *Drosophila*, we have identified the Laminin α1,2; β1; γ1, as the main Laminin required in this process. We show that Laminins are required at different stages of the migratory process, during both early phases of migration, such as the migration into the tail, and late migratory events, such as random and lateral migration over the ventral nerve cord. In addition, laminins are required for the contact-repulsion events that keep an even distribution of macrophages and for lamellipodia formation and stability. Furthermore, our results support the idea that haemocytes secrete the Laminin necessary for their migration as they move.

206A

Role of ADP ribosylation factors and their regulators in collective cell migration. Carlos Zeledon, Xiaojuan Sun, Gregory Emery. Université de Montréal, Montréal, Canada.

Statement of purpose: Cell migration is implicated in various important biological processes, notably morphogenesis and wound healing but it is also central for cancer and formation of metastases. In contrast to single cell migration, collective cell migration implies that cells communicate and maintain their cell-cell adhesion. Recently, our lab has showed that endocytosis regulates cell guidance and cell-cell coordination during collective cell migration. Our hypothesis is that other events of vesicular trafficking might be implicated in collective cell migration. Mainly, we are interested in the role of the small GTPases Arfs, important for the formation of vesicles and sorting of cargo in these vesicles, and their regulators in collective cell migration. Methods and Results: As a model, we use the egg chambers of *Drosophila melanogaster*. Indeed, during oogenesis a group of cells, named border cells, migrate collectively towards the oocyte. By depleting Arfs specifically in borders cells, we produce a strong migration defect. Moreover, three ArfGAPs and one ArfGEF also show a migration defect when depleted. Finally, we found that depletion of Arfs affect various determinant of border cell migration. Whereas, depletion of ArfGAPs affect specifically active receptor tyrosine kinases (RTKs) localisation and the ArfGEF affects solely formation of protrusions. Conclusion and Relevance: Loss-of-function of Arf proteins induce pleiotropic effects, so that it is difficult to determine their exact role during collective cell migration. However their regulators have more specific effects, possibly through the regulation of precise vesicular transport events in the cell. .

207B

A novel role for secreted hemolymph proteins in *Drosophila* muscle attachment. Nicole Green[1], Nadia Odell[2], Clara Bazjek[3], Mitch Dushay[3], Erika Geisbrecht[1]. 1) Bioch. & Mol. Biophysics Dept., Kansas State University, Manhattan, KS; 2) Cell Biol. & Biophysics Dept., University of Missouri-Kansas City, Kansas City, MO; 3) Biology Dept., Illinois Institute of Technology, Chicago, IL.

Stable muscle attachment sites (MASs) are necessary to endure the mechanical stress produced by repetitive muscle contraction. In *Drosophila*, improperly formed and weakened MASs lead to detachment and manifest in mammals as muscle myopathies and/or dystrophies. The dynamic properties of the proteins comprising the extracellular matrix (ECM) mediate muscle contraction and can undergo remodeling to maintain the muscle-tendon association. Fondue (Fon), a secreted ECM protein, was first identified as a constituent of the hemolymph clot in *Drosophila*. We have identified a new role for Fon and other secreted hemolymph proteins at the MAS in *Drosophila* larvae. Using the fusion protein, Fon-GFP, we observed accumulation of Fon at direct and indirect MASs. Subsequent analysis of *fon* mutants revealed body wall muscles that became detached and distinct gapping between muscles. Shared localization patterns and abnormal pupal phenotypes led us to examine a relationship between Fon and another muscle protein, Tiggrin (Tig). Larvae transheterozygous for *fon* and *tig* indicate that these proteins genetically interact at MASs. RNAi knockdown of *tig* in a *fon* mutant sensitized background showed an enhanced detachment phenotype compared to either knockdown alone. This method was used to identify several other secreted hemolymph proteins as components forming the MAS. In addition, transglutaminase (TG), a crosslinking enzyme involved in clot stabilization, was shown to be essential for maintaining muscle attachment. Removal of TG using RNAi caused detachment in L3 fillets. TG protein and the isopeptide bond formed during TG-mediated crosslinking was detected at the MAS using immunofluorescence. We also developed an *in vivo* feeding assay to detect incorporation of a primary amine substrate showing that TG activity occurs at the MAS. These data highlight a discrete set of coagulation proteins that function to provide structural integrity in both coagulation and muscle attachment processes.

208C

A fat body-derived apical extracellular matrix enzyme is transported to the tracheal lumen and regulates tube morphogenesis in *Drosophila*. Shigeo Hayashi[1,2], Dong Bo[1], Guanxia Miao[1,2]. 1) Lab Morphogenetic Signaling, Riken Center Develop Biology, Kobe Hyogo, Japan; 2) Department of Biology, Kobe University Graduate School of Science, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo, 657-8051, Japan.

The apical extracellular matrix (aECM) plays a central role in epithelial tube morphogenesis. In the *Drosophila* tracheal system, Serpentine (Serp), a secreted chitin deacetylase, expressed by the tracheal cells plays a key role in regulating tube length. Here we show that the fly fat body, functionally equivalent to the mammalian liver, also produces Serp protein. Fat body-produced Serp was secreted to the haemolymph, taken up by the tracheal cells, and transcytosed to the lumen to functionally support normal tracheal development. This process was defective in *rab9* and *shrub/vps32* mutants and in wild-type embryos treated with a secretary pathway inhibitor, leading to an abundant accumulation of Serp in the fat body. Our results reveal that the fat body, a mesodermal organ, actively contributes to tracheal development. The regulation of airway morphogenesis by the fat body suggests that this process may play a role in coordinating the size of the developing respiratory system to the dimensions of the insect body.

209A

The Role of Mechanical Cross-linking in Basement Membrane Repair. Angela Howard[1,2], Gautam Bhave[1,3], Andrea Page-McCaw[1,2,4]. 1) Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, Tennessee; 2) Program of Developmental Biology, Vanderbilt University Medical Center, Nashville, Tennessee; 3) Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee; 4) Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee.

The basement membrane is a sheet-like extracellular matrix that wraps around muscle fibers and underlies epithelia. A key component of the basement membrane is type IV collagen, which forms a cross-linked scaffold responsible for much of the mechanical strength of the basement membrane. Collagen IV is sulfilamine-crosslinked at its NC1 domain by peroxidasin, an essential enzyme that resides in the basement membrane. Larvae that have lost the ability to form sulfilimine cross-links develop defects first in the basement membrane of the gut, which appears to lose structural integrity. Because of its high rate of turnover, the normal gut is expected to have significant basement membrane damage requiring an active repair system. We hypothesize that peroxidasin and cross-linking are required for basement membrane repair, which would explain the early appearance of a gut defect in larvae that can no longer form sulfilamine cross-links. We will address the role of peroxidasin during basement membrane repair using an adult gut injury model. In this model, flies are fed Dextran Sodium Sulfate (DSS), which has been observed to induce damage in the gut basement membrane. We then analyze recovery of the basement membrane by quantifying morphological changes in basement membrane characteristics over time. Crosslinking of collagen IV NC1 domains can be blocked by either knocking down peroxidasin or by feeding the flies chemical inhibitors. We will compare the damage and recovery of the gut basement membrane in wild-type flies vs flies unable to crosslink collagen IV NC1 domains. We are also investigating whether peroxidasin is upregulated in gut tissue upon DSS feeding. .

210B

Identification and Analysis of Ras Pathway Candidate Genes. Peter Lyon, Sathiya Manivannan, Ashley Heinaman, Nanki Hura, Molly Josifov, Amanda Simcox. Department of Molecular Genetics, The Ohio State University, Columbus, OH.

Purpose: The oncogenic Ras pathway, which is activated in many human cancers, was first genetically characterized in *Drosophila*. Subsequent genetic screens have identified many additional genes in the pathway. As a complementary approach, we have conducted an *in vitro* screen using an inducible Ras gene in tissue culture cells. Methods: Our lab has developed a conditional *Drosophila* cell line in which proliferation is under the control of oncogenic RasV12 expression induced by GeneSwitch-Gal4 (GSR cells). RNAseq analysis of GSR cells identified 363 genes that were significantly induced by Ras expression. 50 upregulated genes were selected for analysis *in vivo* using RNAi lines from TRiP (Transgenic RNAi Project) and a series of Gal4 drivers. Results and Conclusions: Ubiquitous RNAi expression for each gene driven by *Act5C-Gal4* caused lethality in 16 lines and a wing phenotype in two. Using wing-specific drivers, three showed phenotypes consistent with function in the Ras pathway. We are focusing on *CG4096*, which exhibited a consistent extra vein phenotype. *CG4096* encodes one of the three *Drosophila* ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs). Previous analysis of *CG4096* by our lab has shown that *CG4096* is a negative regulator of the Egfr signaling, though the extent of this interaction has yet to be determined.

211C

Defining the role of Canoe in apical-basal polarity establishment in early Drosophila embryogenesis. Teresa T Bonello[1], Kaelyn Sumigray[2], Mark Peifer[1]. 1) Department of Biology, University of North Carolina, Chapel Hill, NC; 2) Department of Cell Biology, Duke University School of Medicine, NC.

Tissue remodelling is tightly regulated by the actin cytoskeleton. The cytoskeleton allows cells to change shape and move, and also transmits information to cell junctions, facilitating molecular and biophysical cross-talk between cells. We hypothesise that the junctional-actin linker Canoe acts at particular times and in specific tissues during embryogenesis to link cell adhesions to the actin cytoskeleton. Previous data from our lab revealed that Canoe and its regulator Rap1 are essential for initial establishment of apical-basal polarity during Drosophila development. The aim of our research is to identify proteins that regulate and collaborate with Canoe to achieve apical-basal polarity, and to define their mechanisms of action. Our preliminary data indicates Canoe localisation is regulated upstream by Rap1. Canoe is normally restricted to the apical ends of polarising cells during cellularization. Expression of constitutively active Rap1 results in recruitment of Canoe along the entire lateral membrane. Furthermore, preliminary data suggests that a mutant

Canoe protein lacking the Rap1 binding domain localises differentially along the lateral membrane when compared to the full length Canoe protein. Together, this data indicates that Rap1 is required for correct apical positioning of Canoe in the polarising cell. Canoe mutant proteins lacking both the Rap1 and F-actin-binding domain completely fail to localise to the lateral membrane in the developing blastoderm, also implicating actin as an important scaffold for Canoe localisation. Our current research focuses on the mechanism by which Rap1 and the F-actin binding domain mediate Canoe localisation, seeking to identify regulators that act upstream of Rap1 and the effectors on which Canoe acts.

212A

Continuous Talin function is required to maintain apposition of myofibrils between cardiomyocytes. Duygu Cevik[1], Simina Bogatan[1], Abdullah Panchbhaya[1], Valentin Demidov[2], Alex Vitkin[2], J Roger Jacobs[1]. 1) Biology, McMaster University, Hamilton, Ontario, Canada; 2) Medical Physics, University of Toronto, Ontario, Canada.

Muscle insertions, including those of cardiomyocytes, are integrin based adhesions. Integrins, and components of the integrin adhesion complex (IAC) are recycled over time, allowing the insertion to remodel and respond to changes in tension or load. Mechanotransduction of tension can govern the remodeling of cardiomyocytes during growth or cardiomyopathy. We have examined integrin signaling in the Drosophila heart to better understand its role in heart growth and ageing. We have depleted the levels of Talin, a central component of the IAC, at different stages of growth and remodelling of the heart, employing GAL4/GAL80 regulated expression of dsRNA for *rhea*. Depletion of Talin at any stage leads to permanent deficits in cardiomyocyte structure. Most sensitive are the one-to-one apposition of myofibril ends between adjacent cardiomyocytes. Transient loss of Talin expression causes retraction of myofibrils from points of apposition, and myofibrils do not regrow to re-establish apposition once Talin expression is restored. More severe retraction and loss of myofibrils is triggered by transient Talin depletion during remodelling of the heart immediately after hatching and during pupation. In these circumstances, contractile fibres fail to enclose the heart. This phenotype, similar to dilated cardiomyopathy, results in a dilated, fibrillating heart with poor rhythmicity. These experiments highlight the poor regenerative capacity of cardiomyocytes, despite their ongoing capacity to remodel integrin based adhesions.

213B

Phenotypic Analysis of Genes in Retinal Basal Glial cells. Yen-Ching Chang[1,2*], Y. Henry Sun[1,2]. 1) Institute of Molecular biology, Academia sinica, Taipei, Taipei, Taiwan; 2) Department of Life sciences and Institute of Genome sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China.

Retinal basal glial (RBG) cells exist in early development of *Drosophila* visual system at larval stage. The importance of RGB for nerve system has been described in many papers such as helping axon guidance and appropriate wrap of axons. These phenotypes are also the standard and useful features for us to know integrity of visual system. Early in our study, we tried to use different *RNAis* driven by*repoGal4, Mz97Gal4, C527Gal4 and C135Gal4* to find interesting molecular players. Besides, more RBG specific *Gal4* lines have been released. Here, we combine the *RNAi* results with different *Gal4*drivers and analyze the visual system integrity. Different *RNAis* driven by *Gal4* lines (haven't been well-defined) show variable severity of damage to the visual system. Systematic analysis of these phenotypes would help us to understand function of genes in RBG cells.

214C

Biophysical approach to elucidate molecular links between PCP and adhesion/cytoskeleton dynamics. Nabila Founounou[1], Reza Farhadifar[2], Marek Mlodzik[1]. 1) Developmental and regenerative biology, Mt. Sinai School of Medicine , New York, NY; 2) Department of Applied Physics, Center for Systems Biology, Harvard University, Cambridge, MA,.

Planar cell polarity (PCP) refers to the asymmetric distribution of subcellular components within the plane of the epithelium, resulting in polarized morphology and function. In Drosophila eye morphogenesis, a cluster of epithelial cells first differentiate, then rotate 90° as a unit within cells that are immobile/not rotating, in a process termed ommatidia rotation (OR). In mutants that affect PCP, both cell specification and OR direction are affected. Considerable progress has been made in dissecting PCP signaling in cell differentiation. However, how PCP signaling is translated to mechanical forces and how these forces are integrated within the tissue to trigger global changes in tissue shape is unclear. There is evidence indicating crosstalk between PCP and cell adhesion molecules to drive cell movements, but the lack of *in vivo* imaging negatively affected the mechanistic dissection. I developed a novel live-imaging technique to follow OR at the early pupal stage. Unlike previous attempts (using cultured eye discs) this technique is noninvasive and allows collection of dynamic information for several hours in vivo. Using this approach, I started defining a timeline of the onset of different cytoskeletal and junctional proteins asymmetries that will allow elucidating the symmetry-breaking events driving OR. Using live imaging and histological tools combined with computational and quantitative live imaging analysis, the objective is to build a biophysical model of OR based on physiological data which will enable semi-automated identification of PCP-phenotypes mediated by cell adhesion and cytoskeleton defects.

215A

Uncovering a fundamental requirement for septate junction genes during morphogenesis. Sonia Hall, Robert Ward. Molecular Biosciences, University of Kansas, Lawrence, KS.

Morphogenesis is a highly orchestrated tissue level process that is important for development. These cellular events require the precise regulation of signaling pathways to alter cell shapes and allow for cell intercalation to pattern and form complex three-dimensional tissues. While studying imaginal disc morphogenesis, we uncovered a mutation in the gene *Macroglobulin complement*

related (Mcr), which encodes a protein that is a core component of epithelial septate junctions (SJs). The SJ is a protein complex that forms along the apical-lateral membrane and is required to establish a functional paracellular barrier between epithelial cells. Interestingly, loss of *Mcr* not only disrupts this essential paracellular barrier, but also leads to defects in dorsal closure and head involution. We were curious if other SJ genes were similarly required for morphogenesis. We therefore examined over 15 SJ genes for defects in six morphogenetic events using zygotic loss of function alleles or tissue specific RNAi, and determined that every SJ mutant examined showed clear examples of morphogenetic defects in multiple developmental events, revealing a previously under appreciated role for SJ genes in morphogenesis. Surprisingly, the morphogenetic events that require SJ genes occur before the SJ assembles into an occluding junction, at a time when SJ proteins are instead broadly localized along the lateral membrane. From these observations, we propose that SJ proteins serve two distinct functions during development; first, to regulate morphogenesis and then, to form an occluding junction. To gain a mechanistic understanding of the role of SJ genes in morphogenesis, we are studying the function of a subset of SJ genes during dorsal closure to determine if these proteins serve a primary role in signaling, cytoskeletal regulation, or cell adhesion.

216B

An E-Cadherin trafficking defect reveals its role in maintenance of intercellular bridges anchoring. Roland Le Borgne, Nicolas Loyer, Irina Kolotueva. Institut de Génétique et Développement de Rennes, CNRS UMR 6290, Rennes, France.

During gametogenesis of several vertebrate and invertebrate organisms[1] as well as in somatic *Drosophila* epithelial tissues[2], intercellular bridges called ring canals (RCs), resulting from incomplete cytokinesis, exert essential functions in intercellular communication. Despite screens aimed to identify genes involved in RC structure and development, how RCs anchor to the plasma membrane (PM) remains unknown. Here, we report that loss of the clathrin adaptor complex AP-1 in *Drosophila* female germline cysts results in mislocalisation of E-Cadherin from the PM surrounding the RCs into enlarged Rab11-positive endosomes and ultimately detachment of the PM from the RCs. Dominant-negative Rab11 causes similar phenotypes. We further show that this phenotype, also seen in β-catenin mutants, could not be observed in E-Cadherin mutants because N-Cadherin is upregulated, recruits β-Catenin and therefore substitutes for E-Cadherin function. TEM analyses revealed the presence of interdigitated microvilli-like structures that wrap the RCs and are positive for actin, a-actinin and phospho-Moesin. We propose that sustained E-Cad-dependent adhesion supported by AP-1 and Rab11-dependent E-Cadherin trafficking to RCs organizes the actin-based microvilli meshwork and ensures proper attachment of RCs to PM. .

217C

The transmembrane protein Crumbs displays complex dynamics during follicular morphogenesis and is regulated by Moesin, aPKC, the cortical cytoskeleton and endocytosis. Kristin Sherrard, Richard Fehon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The transmembrane protein Crumbs (Crb) functions in apical polarity and epithelial integrity. To better understand its role in epithelial morphogenesis, we examined Crb localization and dynamics in the late follicular epithelium of Drosophila. Crb was unexpectedly dynamic during middle to late stages of egg chamber development, being lost from the marginal zone (MZ) in stage 9 before abruptly returning at the end of stage 10b, then undergoing a pulse of endocytosis in stage 12. This reappearance of MZ Crb is necessary to maintain an intact adherens junction and MZ.

Although Crb has been proposed to interact through its juxtamembrane domain with Moesin (Moe), a FERM domain protein that regulates the cortical actin cytoskeleton, the functional significance of this interaction is poorly understood. We found that while the Crb juxtamembrane domain was not required for adherens junction integrity, it was necessary for MZ localization of Moe, aPKC, and F-actin. Furthermore, Moe and aPKC functioned antagonistically, suggesting that Moe limits Crb levels by reducing its interactions with the apical Par network. Additionally, Moe mutant cells lost Crb from the apical membrane and accumulated excess Crb at the MZ, suggesting that Moe regulates Crb distribution at the membrane, both through interactions with the juxtamembrane domain and through its effects on the cortical actin cytoskeleton. Together, these studies reveal reciprocal interactions between Crb, Moe and the cortical cytoskeleton during cellular morphogenesis.

218A

Tracking vertices in epithelial tissues for the analysis of coordinated cell shape changes during epithelial morphogenesis. Rodrigo Cilla, Steven Del Signore, Victor Hatini. Tufts University, Boston, MA.

Epithelial tissues form cohesive sheets of mechanically interconnected cells. Therefore, mechanical deformation of one cell could affect the cytoskeletal organization, cytoskeletal dynamics and shape of adjacent or distant cells. Most work on epithelial development, however, has focused on the autonomous effects of contractile actomyosin networks on cell shape in process such as apical constriction and cell intercalation. In contrast, much less is known about the non-autonomous effects and supracellular coordination of mechanical forces in epithelial tissue remodeling. To investigate the integration of forces in epithelial morphogenesis we employed the epithelium of the fly eye at late stage of eye development (32-42h APF) as a model. At this developmental stage epithelial cells undergo dramatic cell shape changes without altering their connectivity and therefore must coordinate their mechanical behaviors. Using live imaging and quantitative image analysis we found that eye cellular morphogenesis coincided with oscillations in activity of cytoskeletal proteins, contact length and cell shape. We therefore hypothesize that this oscillatory behavior is used as a mechanism for cells to change their shape and coordinate their mechanical behavior. To rigorously test these ideas, it is necessary to measure cell contacts, cell shapes and molecular dynamics and determine which behaviors are temporally correlated to infer possible cause-effect relationships. A critical

bottleneck for progress in this area has been the lack of image analysis tools that are designed to capture simultaneously the molecular, cellular and tissue level behaviors. Toward a systematic analysis of this problem, we are developing new software designed to automate the segmentation of epithelial cells, identify and track cell vertices, and relate molecular dynamics of force generating molecules with contact dynamics and cell shape changes. We are currently integrating the capability to track molecular dynamics associated with cell contacts and analytics for correlation statistics. These new capabilities will enable us to determine how molecular and mechanical activity in a field of cells integrate to achieve coherent mechanical outputs.

219B

Huntingtin (HTT) transports a novel class of synaptic vesicles during axonal transport: Identification of a moving HTT-Rab4 vesicle in Drosophila larval axons. Joseph A White II[1], Eric Anderson[1], Katherine Zimmerman[1], Kan Hong Zheng[1], Harsh Saxena[2], Ge Yang[2], **Shermali Gunawardena**[1]. 1) Department of Biological Sciences, The State University of New York at Buffalo, Buffalo, NY 14260,; 2) Lane Center for Computational Biology and Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213.

Loss of huntingtin (HTT), the Huntington's disease (HD) protein, causes axonal transport defects. HTT associates with kinesin-1 and dynein motors, but the composition of the moving motor-cargo complex that contains HTT during microtubule (MT) based transport is unknown. Using *in vivo* motility analysis in *Drosophila* larval axons, we identified that HTT influences the motility of a specific subset of Rab-containing vesicles. While reduction of HTT perturbed the bi-directional movement of Rab3, Rab4, and Rab19, and the retrograde movement of Rab7, reduction of HTT stimulated the anterograde movement of Rab2. Dual-color simultanous imaging revealed that Rab4 co-localized with HTT on moving vesicles and in axonal blocks caused by expansion of polyQ. Rab4 co-localized with Rab19, but not with Rab3 vesicles. Rab4 also co-localized with synaptotagmin and synaptobrevin indicating that Rab4 is likely on synaptic vesicles. Collectively, our analysis suggests that HTT differentially regulates the motility of a subset of Rabs. Our results provide direct evidence for a moving HTT-Rab4-motor complex during axonal transport and suggest that disruption of Rab4 transport by mutant HTT could contribute to HD pathology.

220C

Asymmetric mRNA segregation during neuroblast division. Jens Januschke, Anne Ramat. Cell & Developmental Biology, University of Dundee College of Life Sciences, Dundee, United Kingdom.

Mitotic neuroblasts are highly polarized cells that divide asymmetrically roughly every hour to produce two daughter cells of different fate. At each mitosis the cortex gets differentially decorated with cell fate determinants at the apical and basal pole. Then a sophisticated machinery is assembled that controls the orientation of the mitotic spindle to segregate these complexes differentially into the resulting daughter cells. While we have a fair understanding of the molecular details of how cortical protein complexes are assembled, very little is known about the control of the choice of the cortical region that will become apical in mitosis in larval neuroblasts. Hence, which mechanisms ensure the continuity of cell polarity orientation between different cell cycles?

We have previously found a role for centrosomes and the interphase microtubule network in this process but their precise contribution remains unclear. One interesting possibility is that the microtubule network positions cues that help choose the place where cortical crescent formation is initiated.

Intriguingly many transcripts of genes that are involved in asymmetric cell division have been reported to localize to the apical side of neuroblasts. Given the tight time constraints of the neuroblasts cell cycle, we reasoned that spatially controlled translation of localizes mRNAs might contribute to establish cell polarity in neuroblasts.

How these mRNAs are maintained apically and if their localization contributes to establishing neuroblast polarity remains unclear. To systematically address the level of polarized transcript localization in a stem cell we are performing an mRNA localization screen in larval neuroblasts. We will report on a surprising localization of one transcript during the larval neuroblasts cell cycle. We followed mRNAs in living neuroblast by VIDEO microscopy and summarize what we have learned so far about the mechanism and function of this process during asymmetric neuroblast division.

221A

Transmembrane and secreted MMPs are required for heart morphogenesis in *Drosophila*. Qanber S Raza, Jessica Vanderploeg, Roger Jacobs. McMaster Univeristy, 1280 Main St West, Hamilton, ON,CA L8S4L8.

Matrix Metalloproteinases (MMPs) are enzymes which break down components of the extracellular matrix (ECM) and promote many homeostatic and disease related processes. Their role in epitheliliazation, migration and tumour invasion has been extensively described. Much less is known about their function during embryogenesis. Due to the high level of redundancy between the 24 mammalian MMPs, *Drosophila*, whose genome contains two copies of *mmp* genes MMP1 and MMP2, provides an attractive genetic model. Other than branching morphogenesis, previous studies have not uncovered essential *in vivo* functions for MMPs during embryogenesis. We have explored the role of MMPs during embryonic heart development as this simple organ undertakes cell migration and ECM synthesis during early morphogenesis. To form a functional heart, ventrally specified cardioblasts (CB) must collectively migrate to the dorsal midline, fuse with the contralateral partners, and subsequently form a lumen. We performed fluorescent live imaging to examine collective cardioblast migration in embryos mutant for MMP1 and/or MMP2. Our time-lapse movies indicate that both the secreted and membrane-attached MMPs (MMP1 and MMP2) are essential for CB outgrowth formation and lamellipodial activity at the leading edge. However, only MMP2 is essential for dorsal and ventral adhesion formation between contralateral CBs. Subsequent lumen formation as well requires MMP2 activity, whereas absence of MMP1 activity leads to reduced lumen size. In addition, migration velocity and the integrity of bilateral CB rows were significantly affected in both MMP mutants

compared to wildtype. Ectopically inhibiting MMPs by overexpressing Tissue Inhibitors of Metalloproteinase (TIMP) in the surrounding ectoderm also resulted in a no lumen phenotype. When monitored through collagenIV-GFP distribution, ECM deposition was misregulated in individual MMP mutants. Moreover, in MMP double mutants, collagenIV-GFP levels were virtually undetectable. Overall, we demonstrate the first embryonic requirement of MMPs.

222B

Expression of the testis-specific paralog of ATP synthase subunit d in *Drosophila* flight muscle. Dennis Akrobetu, Brian Johnson, Lindsay Regruto, Eric Sawyer, Karen Hales. Department of Biology, Davidson College, Davidson, NC.

ATP synthase generates ATP through the chemiosmotic flow of protons down their gradient during electron transport. Besides its role in cellular respiration, ATP synthase participates in dimerization and oligomerization which lead to the sharp curvatures of the mitochondrial cristae. Previous work has suggested the key role of subunit d of the complex to these dimerization events. During *Drosophila melanogaster* spermatogenesis, mitochondria undergo a series of shape changes which include the formation of the novel organelle known as the nebenkern. Aberrant clumping of the nebenkern is displayed by *ms(2)*1400 mutants during spermatogenesis. *CG7813* encodes a testis-specific paralog of ATP synthase subunit d and is the annotated gene associated with *ms(2)1400*. CG7813 is unusually large and may hinder ATP synthase dimerization, allowing nebenkern development in the testis. This effect of *CG7813* may be deleterious when expressed in other tissues by causing aberrant cristae morphology. The UAS-GAL4 system was used to overexpress *CG7813* in *Drosophila* flight muscle. *UASCG7813-GFP/CyO;Act88F-GAL4(III)* flies were compared to *UASmito-GFP/CyO;Act88F-GAL4(III)* control flies for evidence of mitochondrial fragmentation. Insertion of a tagged *UASmito-GFP* gene and an untagged *UASCG7813* gene into one fly strain revealed that preliminary observations of mitochondrial fragmentation were due to the localization pattern of CG7813. Continuing research will aim to detect if the ectopic expression of *CG7813* causes any subtle deviations in flight muscle function. .

223C

F-box proteins in *Drosophila* muscle. C. Clark[1], K. Bauman[2], M. Kelly[2], E. Geisbrecht[1]. 1) Dept. of Biochem. & Mol. Biophysics, Kansas State University, Manhattan, KS; 2) Cell Biology & Biophysics Dept., University of Missouri KC, Kansas City, Mo.

Balancing protein synthesis and protein degradation is important for normal cellular function and the prevention of disease, including muscle atrophy. The well-studied ubiquitin-proteasome pathway is one route for selective protein degradation. Three enzymatic protein components, an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-ligating enzyme, are all necessary to conjugate ubiquitin molecules to the target substrate for subsequent degradation by the proteasome. F-box proteins (FBP) contain a 40 amino acid F-box domain and recruits substrates for the Skp1-cullin1-FBP-type E3 ubiquitin ligases. In *Drosophila melanogaster*, there are 28 F-box proteins, of which the majority are uncharacterized. We have focused on a subset of F-box proteins that are expressed in the developing embryonic musculature that may be essential to regulate protein turnover. One of these, CG5961, is orthologous to the uncharacterized vertebrate F-box only protein 9 (Fbxo9). Both CG5961 and Fbxo9 contain N-terminal tetratricopeptide repeat-containing (TPR) repeats and an internal F-box domain. We hypothesized that loss of CG5961 function, or muscle-specific inactivation of the F-box region (ΔF-box), would result in muscle defects if target proteins are not properly degraded. Conversely, over-expression of full-length CG5961 protein levels may also perturb muscle patterning due to a decrease in target substrate protein levels. Consistent with these predictions, embryonic muscle defects were observed when CG5961 function was decreased by RNAi or altered by expressing transgenic flies that express the ΔF-box form of CG5961. In addition, increased levels of full-length CG5961 also resulted in aberrant muscle patterning. An additional line of investigation involves identifying potential muscle-specific target substrates for the E3 ubiquitin-ligase activity of CG5961 complexes. We have developed an *in vivo* method to isolate and identify potential target substrate proteins in muscle tissue. Future research will include both genetic and biochemical approaches to validate the regulation of these substrate targets by CG5961. .

224A

The Role of Ubiquitin Specific Protease 5 in *Drosophila melanogaster*. Gorica Ristic, Sokol Todi. Pharmacology, Wayne State University School of Medicine, Detroit, MI.

The covalent attachment of a ubiquitin molecule onto a substrate protein, referred to as ubiquitination, affects protein stability, localization and function and thereby serves important roles in numerous cellular pathways. Proteases known as deubiquitinating enzymes (DUBs) are responsible for carrying out the reversal of ubiquitination and in turn regulate various cellular processes ranging from gene transcription to protein degradation. Ubiquitin Specific Protease 5 (USP5) is a member of the Ubiquitin Specific Protease subclass of DUBs. This DUB is reported to hydrolyze unanchored (free) poly-ubiquitin chains and to recycle mono-ubiquitin for re-utilization in the cell. However, the physiological role of USP5 *in vivo* is not entirely clear. *Drosophila* USP5 is well conserved compared with its human counterpart. Through *in vitro* biochemical reactions we show that *Drosophila* USP5, similar to the human orthologue, is able to cleave specific types of ubiquitin-ubiquitin linkages rapidly. Additionally, both the human and the fly USP5 quickly disassemble both unanchored poly-ubiquitin chains as well as ubiquitinated substrates *in vitro*. This DUB is developmentally required in the fly: knockdown of USP5 throughout the fruit fly results in lethality during development, and tissue-specific knockdown shows that USP5 is also important in glial and muscle cells as well in the fat body. At the biochemical level, knockdown of USP5 results in the accumulation of poly-ubiquitin species without a depletion of mono-ubiquitin. Based on further genetic, biochemical and physiological assays, USP5 leads to lethality potentially due to the accumulation of poly-ubiquitin chains, rather than depletion of mono-ubiquitin, which perturbs various cellular processes. Ongoing studies aim to pinpoint the precise physiological function of this DUB in *Drosophila*..

225B

Characterization of a novel Wnt signaling factor regulating tissue homeostasis and regeneration in the aging *Drosophila* intestine. Anna-Lisa Boettcher, Teresa Eichenlaub, Claudia Strein, Michael Boutros. German Cancer Research Center (DKFZ), Division of Signaling and Functional Genomics and Heidelberg University, D-69120 Heidelberg, Germany.

Tissue homeostasis of highly regenerative adult organs, like the gut epithelium, is maintained by a small population of stem cells, which reside within the tissue. They continuously divide to self-renew and to generate differentiated progenies, a process that is controlled and regulated by signals coming from the surrounding cells. A critical mediator of signaling events during adult homeostasis and development is the highly conserved Wnt family of secreted proteins that activate different downstream signaling cascades. *Drosophila melanogaster* provides a powerful genetic model system for studying conserved regulatory mechanisms and has advanced our understanding of signaling transduction pathways in humans. In the *Drosophila* midgut, regeneration of the intestinal epithelium is remarkably similar to regeneration of the mammalian midgut, making it also an ideal model system to examine the change of tissue functionality during the process of aging.

Using RNA interference (RNAi) *in vivo*, we have identified potential novel signaling components, which are required for the maintenance of intestinal stem cells (ISCs) as well as responsible for their reduced ability in aged flies to produce newly differentiated tissue cells, leading to a decreased tissue regeneration. With this approach we have uncovered a number of potential novel signaling components, among them was a novel gene that putatively plays a role in the regulation of Wnt signaling. The depletion of that gene in the ISCs of the fly midgut, significantly increased the number of stem and progenitor cells within the epithelium, similar to the phenotype observed when knocking down *Apc*, a known negative regulator of the Wnt pathway. Based on these findings, we called the gene *enhancer of stem cells (eos*). Our results propose that *eos* plays a role as a secreted negative regulator of Wnt signaling, thus might be required for tissue homeostasis and epithelial regeneration in the aging *Drosophila* intestine.

226C

Ras activated Dsor1 promotes Wg signaling in *Drosophila* development. Eric T Hall, Esther M. Verheyen. Simon Fraser University, 8888 University Dr. Burnaby, BC, Canada V5A 1S6.

Wnt/Wingless (Wg) signaling plays a fundamental role in growth, cell-fate determination, and adult tissue homeostasis. This is achieved by extensive communication with other signaling pathways, leading to synergistic or antagonistic effects normally resulting in desirable biological outcomes. The Wg signaling cascade is tightly regulated at multiple levels. Regulation occurs through a myriad of phosphorylation events to promote or inhibit signal transduction. A comprehensive *in vivo* RNAi screen of the kinome and phosphatome in *Drosophila* performed in our lab identified several components of the Ras/MAPK signaling cascade as modulators of Wg target genes. Thus far, several and often conflicting modes of interaction between Ras/MAPK and Wg signaling have been identified in specific cellular contexts. Examination of Wg pathway activity in *Drosophila* imaginal discs revealed genetic evidence of a novel interaction in which the *Drosophila* dual specific kinase MEK, Downstream of Raf1 (Dsor1), is needed for Wg signal transduction. Knockdown of Dsor1 resulted in loss of Wg target gene expression, as well as reductions in stable Armadillo (Arm; *Drosophila* β-Catenin) levels in wing discs. Introduction of a catalytically inactive Dsor1 caused levels of active β-Catenin to decrease. Analysis of Dsor1 using PLA identified a close physical interaction with Arm. These results suggest that Dsor1 normally counteracts the destruction complex and may promote its membrane recruitment. The recruitment and inactivation of the destruction complex is essential for the stabilization of newly synthesized Arm, which then enables it to promote transcriptional activation of target genes. As part of the MAPK signaling cascade, Dsor1 requires activation via upstream signaling components. In the developing imaginal discs one of the few identified initiators of the Ras/MAPK cascade is EGFR. We have identified that Ras/Dsor1 activity is independent of EGFR, and appears to utilize the insulin receptor for activation in promoting Wg signaling. Together our results suggest novel crosstalk between Insulin and Wg signaling in the developing *Drosophila* via Dsor1 recruitment of the destruction complex to the membrane.

227A

Testing models of the APC tumor suppressor/β-catenin interaction reshapes our view of the destruction complex in Wnt signaling. David Roberts, Robert Yamulla, Eric Kane, Alexandra Moody, Kristin Politi, Nicole Lock, Andrew Foley. Department of Biology, Franklin & Marshall College, Lancaster, PA.

The Wnt pathway plays important roles in normal development and homeostasis, but is also misregulated in human diseases such as cancer. Adenomatous polyposis coli (APC) is an essential negative regulator of Wnt signaling inactivated in the vast majority of all colorectal cancers. APC participates in a multiprotein “destruction complex” that targets the key effector of Wnt signaling, ß-catenin, for ubiquitin-mediated proteolysis. Several fundamental questions remain regarding how the destruction complex works as a molecular machine including: what is the mechanistic role of APC in the destruction complex? Several models of APC function have recently been proposed based on compelling biochemical data. Many of these models have emphasized the importance of phosphorylation of high-affinity ß-catenin-binding sites (20-amino-acid repeats, 20Rs) on APC. Here we test these models by generating a Drosophila APC2 mutant lacking all ß-catenin-binding 20Rs and performing functional studies in human colon cancer cell lines and Drosophila embryos. Our results are inconsistent with proposed models, as we find that the 20Rs of APC are not required for destruction complex activity. We also generate an APC2 mutant lacking all ß-catenin-binding sites (15Rs and 20Rs) and find that a direct ß-catenin/APC interaction is surprisingly not essential for ß-catenin destruction, although it does appear to increase destruction complex efficiency in certain developmental contexts. Overall, our findings support a model whereby ß-catenin-binding sites on APC do not provide a critical mechanistic function per se, but rather dock ß-catenin in the destruction complex to increase the efficiency of ß-catenin destruction.

Furthermore, in Drosophila embryos expressing some APC2 mutant transgenes we observe a separation of ß-catenin destruction and Wg/Wnt signaling outputs and suggest that cytoplasmic retention of ß-catenin likely accounts for this difference.

228B

Characterization of a conserved, negative regulator of Wg/Wnt signaling in *Drosophila melanogaster*. Michael Suchanek, Varun Chaudhary, Gerrit Erdmann, Michael Boutros. German Cancer Research Center (DKFZ), Div. Signaling and Functional Genomics and University of Heidelberg, Department of Cell and Molecular Biology, Faculty of Medicine Mannheim, D-69120 Heidelberg, Germany.

Tissue growth, polarity and patterning, development and differentiation are complex processes controlled by numerous signaling cascades. One crucial mediator of signaling events during development and tissue patterning is the highly conserved Wnt family of secreted protein (Wnt) signaling cascade. Although the Wnt/*wingless* (*wg*) pathway has been extensively studied over the past years not all regulatory networks and downstream mechanisms of the pathway have been unraveled.

Using RNAi *in vivo* in *Drosophila,* we identified a gene "*Regulator of wg" (Rew),* which is important during wing development and has previously been reported to be involved in border cell migration. RNAi-mediated silencing of *Rew in vitro* significantly increased Wnt reporter activity and knock-down in the wing disc resulted in up-regulation of the long-range *wg* target gene *distal-less (dll)*. Its depletion in the complete wing disc using *nubbin-Gal4* resulted in the induction of supernumerary ectopic chemosensory bristles at the wing margin. Furthermore, overall wing size was significantly reduced compared to the wildtype wing suggesting the involvement of *Rew* in other growth regulating pathways.

Similar to *Drosophila*, silencing of *Rew* in human cell lines increased Wnt reporter activity and lead to an up-regulation of the Wnt target gene Axin2 on both mRNA and protein level.

In conclusion, we have discovered a novel repressor of Wnt signaling, which is conserved between flies and humans. It seems to play a crucial role during the development of various tissues and cell types balancing Wnt activity and regulating cell-fate decisions. .

229C

Sol narae (Sona) is required for cleavage of exosomal Wingless and generation of a soluble Wingless fragment. Jong-Hoon Won[1], Go-Woon Kim[2], Ja-young Kim[1], Ok-Kyung Lee[1], Sang-Soo Lee[1], Orkhon Tsogtbaatar[1], Su-Jin Nam[1], Yeon Kim[1], Kyung-Ok Cho[1], Go-Woon Kim. 1) Biological Science, KAIST, Daejeon, South Korea; 2) Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Korea.

Wnt family proteins are secreted morphogens essential for embryonic development and adult homeostasis in all metazoans. Mechanisms for the secretion of Wnt and composition of extracellular Wnt are, however, still largely unknown. Here, we show that a fly ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) named Sol narae (Sona) is involved in generating an active form of soluble extracellular Wg, a mammalian homolog of Wnt1, and thus promotes Wg signaling. Sona and Wg specifically colocalized in early endosomes and were enriched on exosomes. Interestingly, Wg fragments were generated on exosomes only in the presence of protease-active Sona, demonstrating that Sona's protease activity is essential for cleaving Wg. The cleavage separates the soluble C-terminal domain (CTD) from the membrane-bound N-terminal domain (NTD) of Wg. The CTD was uniquely abundant in the region distant from Wg-producing cells. Given that the CTD has full Wg activity, we propose that Sona is required for the formation of soluble CTD that can travel faster than other forms of Wg.

230A

Latency of a Drosophila BMP-prodomain complex is prevented by proconvertase cleavage within the prodomain. Edward Anderson, Eric Tung, Kristi Wharton. MCB, Brown University, Providence, RI.

Bone Morphogenetic Proteins (BMPs) are cleaved from the highly conserved C-terminal domain of a proprotein dimer by proconvertases. In some cases, the resulting prodomain and ligand domain remain associated and are secreted as a complex. While the presence of the TGFβ prodomain renders the ligand "latent" and inaccessible to its receptor, BMP ligand-prodomain complexes retain activity (Sengle et al. 2011). Our lab found that the Drosophila BMP5/6/7/8 orthologue Gbb can undergo alternative processing to produce two ligand variants (Akiyama, Marques, and Wharton 2012). Cleavage at the S1 site produces Gbb15, a short form composed only of the conventional C-terminal ligand domain, while cleavage at the NS site produces Gbb38, a larger ligand composed of roughly half of the prodomain and the C-terminal ligand domain. In vivo analyses indicate that Gbb38 exhibits a greater range and activity than Gbb15. A third site, S0, has been identified as redundant with S1 (Fritsch et al. 2012). Furthermore, the relative abundance of Gbb15 and Gbb38 varies between tissues, suggesting that cleavage site preference may be a regulated process.

Our current studies indicate that Gbb is secreted in complex with its prodomain cleavage products. Mutations at the NS or S1 cleavage site reduce signaling activity. Mutant Gbb ligands are also secreted in complex with their respective prodomain cleavage products. Mutations of the NS site increase ligand abundance and affinity of the prodomain for Gbb15. S0 cleavage is inefficient, as when the S1 site is mutated partially cleaved dimers are secreted. Overall, our results are consistent with a model where cleavage of the NS prodomain site prevents "latency" of the Gbb15-prodomain complex.

231B

A DPP-mediated Feed-Forward Loop canalizes morphogenesis during *Drosophila* dorsal closure. Antoine Ducuing[1], Charlotte Keeley[2], Bertrand Mollereau[1], Stéphane Vincent[1]. 1) Laboratory of Molecular Biology of the Cell, Ecole Normale Supérieure de Lyon, Lyon, France; 2) Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.

Development is robust since nature has selected various mechanisms to buffer the deleterious effects of environmental and genetic

variations to deliver phenotypic stability. However, how cells interpret and integrate different signals to ensure robust signal transduction and therefore robust development remains poorly understood. Dorsal closure (DC) in the *Drosophila* embryo is an elegant system to understand signal integration: hundreds of leading edge (LE) cells differentiate and act in concert to seal the dorsal opening in a process reminiscent of wound healing. Strikingly, two major developmental pathways are active in LE cells: the stress response pathway JNK acts upstream and induces the Bone Morphogenetic Protein homologue DPP. Both JNK and DPP signaling pathways are crucial for DC since embryos mutants for either JNK or DPP pathway components fail to close dorsally and exhibit a dorsal open phenotype. However, how JNK and DPP contribute to DC and how the signals are integrated in a robust manner remain a conundrum for more than 17 years. Here we report that DPP and JNK form a coherent Feed-Forward Loop (FFL) that controls the specification and differentiation of leading edge cells during *Drosophila* dorsal closure. We provide molecular evidence that through repression by Brinker, the DPP branch of the FFL filters unwanted JNK activity. High-throughput live imaging revealed that this DPP/Brk branch is dispensable for dorsal closure under normal conditions but is required when embryos are subjected to thermal stress. Our results indicate that the wiring of DPP signaling buffers against environmental challenges and canalizes cell identity. We propose that the main function of DPP pathway during *Drosophila* dorsal closure is to ensure robust morphogenesis, a distinct function from its well-established ability to spread spatial information. .

232C

Dpp signaling antagonism is mediated by the formation of a chondroitin sulfate sink. Matthew Moulton, Gregory Humphreys, Anthea Letsou. Human Genetics, University of Utah, Salt Lake City, UT.

Glycosylation has been identified as a mechanism of protein regulation in a growing number of contexts, of which Drosophila embryogenesis is no exception. Our lab and others have shown that Dpp signaling is regulated by glycosylation during development, and our lab has characterized the role of *mummy* (*mmy*) in a unique form of Dpp signal antagonism dependent upon chondroitin sulfate (CS). *mmy* encodes the enzyme required for the last step in the synthesis of UDP-*N*-acetylglucosamine (UDP-GlcNAc), a sugar used for glycosylation and synthesis of glycosaminoglycans like CS proteoglycans. *mmy* mutants exhibit hyperactive Dpp signaling cuticle phenotypes, as well as molecular phenotypes that include expanded embryonic epidermal domains of both pMad and *dpp* consistent with a role for *mmy* in Dpp signal antagonism. To better understand the mechanism by which glycosylation is used to antagonize Dpp signaling and with the expectation that enzymes required for GlcNAc utilization and Dpp signal antagonism will exhibit shared loss-of-function phenotypes with *mmy*, we carried out an RNAi screen targeting all transferases involved in GlcNAc utilization. Here we present data showing that one of the transferases identified in this screen, the chondroitin sulfate synthase *wanderlust* (*wand*), is required for the creation of a localized Dpp sink. From its position close to the Dpp signaling source, this sink limits signaling to the dorsal-most domains of the epidermis during dorsal closure. Our data suggest a novel role for glycosylation in maintaining the proper Dpp signaling range in the embryonic epidermis and provide the first evidence for a localized Dpp sink.

233A

Uncovering a role for the TGF-β/Activin factor Myoglianin in regulating wing imaginal disc growth through analysis of dSmad2 protein null mutants. Ambuj Upadhyay, Aidan J. Peterson, Michael B. O'Connor. Dept. of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

How organ size is determined is still an active question in developmental biology. We are using the Drosophila wing imaginal disc as a model to identify novel genes and mechanisms which finely tune this process. In the wing imaginal disc, Transforming Growth Factor β (TGF-β) signaling plays a crucial role regulating organ size. Decapentaplegic (Dpp), a member of the TGF- β superfamily of ligands and homologous to vertebrate Bone Morphogenic Protein BMP2/4, is secreted to form a gradient within the wing epithelium that is important for both the growth and patterning of the tissue.

In our studies of the less well characterized TGF-β/Activin pathway, we observed that a protein null mutation for dSmad2 results in a disc overgrowth phenotype that is similar to a gain in Dpp signaling. The overgrowth requires Babo, the Activin receptor, along with key components downstream of the Dpp receptor (Mad and Schnurri), suggesting a cross talk between the Activin and BMP pathways. Here we demonstrate that the dSmad2 null mutant overgrowth phenotype requires a specific Activin-type ligand (Myoglianin, Myo), a specific Type-I receptor isoform (Babo-a), and a specific Type-II receptor (Punt). Using genetic epistasis analysis we demonstrate that Babo-a isoform (but not Babo-b/c) and Punt (but not Wit) are epistatic to dSmad2. We propose that Myo may be supplied systemically and signaling via Babo-a to regulate wing disc growth. We are currently exploring the relevant tissue source for Myo. Furthermore, we are characterizing the role of Myo in disc growth and patterning in wild type animals.

In summary, our work has identified novel functions of Myoglianin in wing disc growth control. We are working towards understanding the molecular mechanisms of how Myo regulates growth and patterning in the wing disc since this is crucial for our broad understanding of organ size regulation in Drosophila and higher vertebrates.

234B

Dissecting the nuclear co-repressor complex(es) of Notch. Stephen Chan, Gustavo Cerda-Moya, Bettina Fischer, Robert Stojnic, Sarah Bray. Anatomy Building, Department of Physiology, Development and Neuroscience, Cambridge University, Cambridge, United Kingdom, CB2 3DY.

Contact dependent Notch signaling regulates numerous important processes such as cell proliferation, differentiation and survival. Indeed, the inappropriate activity of Notch contributes to diseases including various types of cancer. One of the current unsolved questions is how the Notch pathway regulates different genes in a context dependent manner. The core transcription factor, Su(H) in

Drosophila, is known to associate with a diverse set of co-repressors, and it is thought that Notch activity promotes gene expression by displacing these co-repressor complexes. The relevance of the composition and function of the different co-repressor complexes to the specificity of the Notch responses remains however poorly understood. To investigate further the mechanisms, we have conducted genome-wide ChIP to ascertain the binding profiles of three co-repressors that have been linked to Su(H) functions: Smr, ebi and Hairless. Each of these exhibits different genome-wide profiles in third instar larval wing discs that overlap with Su(H) to variable extents. From these overlaps, enhancers can be categorized into different sub-types. The relevance of these to Notch sensitivity is currently being explored. .

235C

The Ecdysone and Notch pathways regulate Cut via a shared cut enhancer region for proper dorsal-ventral (D/V) boundary formation in Drosophila wing disc. Allison Jevitt, Dongyu Jia, Jamal Bryant, Wu-Min Deng. Florida State University, Tallahassee, FL.

The development of metazoan organisms requires coordination of complex regulatory pathways in order to regulate patterns of gene expression. The Drosophila imaginal wing disc is the ideal model system for the study of gene interactions regulating cellular growth and development. Notch signaling has been known to regulate Cut expression in wing discs for the D/V boundary formation. Our recent findings from gain and loss of function studies have identified that another factor, Broad (Br), a zinc-finger transcription factor, is also involved in regulating Cut expression in the DV boundary region. However Br expression is not regulated by Notch signaling in wing discs, raising the possibility that another upstream signal is required for the Br-dependent regulation of Cut expression. Here, we report that Ecdysone signaling is the upstream signal to induce Br for the upregulation of Cut expression in D/V boundary of the wing disc. This novel evidence suggests that Notch and Ecdysone pathways act in parallel to upregulate Cut for proper D/V boundary formation. Regarding the mechanism underlying upregulation of Cut via Ecdysone-Br cascade, we found that Ecdysone signaling could upregulate Cut-lacZ expression, a reporter containing a 2.7 kb cut enhancer region. Moreover, within this 2.7 kb enhancer region, we identified canonical Su(H) and Br binding sites, suggesting that Notch and Ecdysone pathways directly regulate cut at these enhancer region via their transcription factors, Su(H) and Br, respectively. These findings, taken together, suggest a novel model that Notch-Su(H) and Ecdysone-Br signaling cascades, might work synergistically to induce Cut expression for proper D/V boundary formation in the wing disc.

236A

Deciphering the Function of *midline* within Insulin/dAKT and c-Jun N-terminal Kinase Signaling Pathways during Larval CNS and Eye Development. Sandra Leal, Q. Brent Chen, Visic Petra, Buford Ken. Dept Biological Sci, Univ Southern Mississippi, Hattiesburg, MS.

We recently reported that the T-box transcription factor *midline* (*mid*) functions within the Notch-Delta signaling pathway to specify sensory organ precursor (SOP) cell fates in early-staged pupal eye imaginal discs and to suppress apoptosis (Das et al., 2013). From genetic and allelic modifier screens, we now report that *mid* interacts with genes downstream of the insulin receptor(InR)/Akt and c-Jun-N-terminal kinase signaling pathways (JNK) to regulate interommatidial bristle (IOB) formation and cell survival. In common with Notch signaling, both the InR/Akt and JNK pathways are responsive to physiological stress and are functionally linked to p53-mediated apoptosis. One of the most significant *mid*-interacting genes identified from the modifier screen is dFOXO, a transcription factor exhibiting a nucleocytoplasmic subcellular distribution pattern. In common with dFOXO, we show that Mid exhibits a nucleocytoplasmic distribution pattern within WT third-instar larval (3^{o}L) tissue homogenates. Because dFOXO is a stress-responsive factor, we are currently assaying the effects of either oxidative or metabolic stress responses on modifying the *mid* mutant phenotype which is characterized by a 50% loss of IOBs within the adult compound eye. We are also immunolabeling WT and *dFOXO* null 3^{o}L eye discs and brains with anti-Mid antibody to determine whether *dFOXO* is required to regulate Mid expression. Taken together, these studies will assess whether Mid and dFOXO serve as critical effectors of cell fate specification, differentiation, and survival within Akt and JNK signaling pathways during 3^{o}L development. .

237B

The *Drosophila* glucoside xylosyltransferase Shams is a novel ligand-specific regulator of Notch signaling. Tom Lee[1], Hamed Jafar-Nejad[1,2]. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Program in Developmental Biology, Baylor College of Medicine, Houston. TX.

Glycosylation plays an important role in modulating the function of Notch(N). The protein *O*-glucosyltransferase Rumi promotes N signaling by adding *O*-glucose onto N. The *O*-glucose can be extended by the addition of xylose residues by the glucoside xylosyltransferase Shams. We have reported that xylosylation of N by Shams inhibits N signaling in certain contexts including wing vein formation, and that loss of Shams in the pupal wing disc results in increased N cell surface expression. However, the *shams* loss-of-function phenotypes cannot be fully explained by altered N expression. Based on the cell-type specific effects in *shams* mutants, we hypothesized that Shams inhibits N activation by Delta(Dl) but not Serrate(Ser). Providing additional genomic copies of *Dl* but not *Ser* enhances the wing vein loss in*shams* mutants and in animals harboring a *N* transgene with mutations in the functional xylosylation sites. In addition, *shams* mutations can suppress *Dl* haploinsufficient phenotypes and *vice versa*, but removal of one copy of *Ser* does not alter the *shams* mutant phenotype. Removing one copy of *shams* also suppresses the mutant wing phenotypes caused by knock-down of the GlcNAc transferase*fringe*. Moreover, loss of *shams* results in an increase in N *trans*-activation induced by ectopic expression of Dl, but not ectopic Ser. These observations indicate that Shams opposes *trans*-activation of N by Dl. Cell aggregation assays indicate that Shams inhibits binding of Dl and N *in trans* but has no effect on binding between Ser and N. Interestingly, the

effects of Shams loss on the ability of overexpressed Dl to *trans*-activate N can be phenocopied by removing one copy of *Dl* and/or *Ser*, indicating that loss of Shams mimics a relative decrease in the *cis*-inhibition of N by ligands. Taken together, these data indicate that Shams is a ligand-specific modulator of N signaling and suggest that xylose residues on N help determine the balance of N receptor's response to *cis*- vs. *trans*-Dl by altering N/Dl binding. Moreover, the data suggest that Shams and Fringe play a combinatorial role in modulating N activation.

238C

A screen for tyrosine phosphatases redundant with Eyes absent. Charlene Hoi, Trevor Davis, Ilaria Rebay. Ben May Department of Cancer Research, University of Chicago, Chicago, IL.

Eyes absent (Eya) is a central member of the retinal determination gene network (RDGN), which is essential in mediating *Drosophila* eye development. Classically, Eya has been placed in the network as a transcription co-factor that works together with Sine oculis (So) to induce transcription of target genes; however, other functions for Eya may exist. Eya can be localized to the cytoplasm upon phosphorylation by Abelson, and Eya family members from plants to humans exhibit protein tyrosine phosphatase (PTP) activity in vitro. While a variety of Gal4/UAS-based assays have suggested a requirement for Eya in the cytoplasm as a PTP, its substrates and specific functions in this context remain elusive. A recent study from Graeme Mardon and colleagues reports the surprising discovery that Eya's PTP is not required for normal development (Jin et al, PLoS ONE, 2013). Although one interpretation of their result is that *Drosophila* Eya lacks PTP activity in vivo, an alternate hypothesis is that Eya's PTP activity may be redundant with that of other tyrosine phosphatases. We have designed a genetic screen to test this latter possibility and will report the results here.

239A

Exploring the Relationship Between ETS-Family Transcription Factor Polymerization and RTK Signaling Output. Matthew Hope[1], John Reinitz[2], Ilaria Rebay[2]. 1) Department of Biochemistry and Molecular Biophysics; 2) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Receptor Tyrosine Kinase (RTK) signaling is deployed in multiple contexts throughout *Drosophila* development, resulting in activation of the canonical Ras/Raf/MAPK cascade and post-translational modification of downstream transcription factors (TFs), such as Yan. In some contexts, such as R7 photoreceptor specification, the level of activated MAPK is read by cells in order to overcome differential thresholds of transcriptional repression, but the molecular basis of these thresholds is unknown. The question of how cis-regulatory element architecture and TF-TF interactions interface to establish differential responses to activated MAPK is not well understood, but potentially critical for understanding RTK signaling in many contexts throughout development. Yan is a polymerizing member of the ETS-family of transcription factors, and phosphorylation of Yan by activated MAPK results in both depolymerization of Yan and derepression of its target genes. We speculate that the strength of Yan polymerization might be precisely tuned to set the threshold for activation by MAPK, and we are testing this hypothesis using an approach that combines biochemistry, genetics, and mathematical modeling. We have developed a model of Yan polymerization based on statistical mechanics, which reveals cooperative occupancy of sites by Yan as a function of different polymerization affinities. To validate the predictions of our model, we leveraged structural information about Yan's polymerization domain, the SAM domain, to generate a suite of missense mutants of Yan that increase and decrease the strength of SAM-SAM interactions relative to wildtype Yan. Using an *in vitro* native gel shift assay, we have shown that the point mutations demonstrate a wide range of polymerization affinities, and are confirming these behaviors in single-cell nuclear FRAP assays. Ongoing work is centered upon creating mutant polymerization alleles in the endogenous locus in order to determine their effect on transcriptional repression, MAPK sensitivity, and ultimately cell fate decisions.

240B

Cellular and temporal dynamics of the Pointed-Yan network during cell fate specification in the Drosophila eye. Jean-Francois Boisclair Lachance[1,3], Nicolás Peláez[2,3], Luís Amaral[2,3], Richard Carthew[2,3], Ilaria Rebay[1,3]. 1) University of Chicago, Chicago, IL; 2) Northwestern University, Evanston, IL; 3) Chicago Center for Systems Biology (CCSB), Chicago, IL.

Two ETS family transcription factors, Pointed (Pnt) and Yan, mediate the transcriptional response downstream of receptor tyrosine kinase (RTK) signaling to direct cell fate specification in the fly retina. The Pnt-Yan network has been modeled as a bistable switch in which RTK signaling triggers differentiation by inducing transitions from a high Yan/low Pnt to a low Yan/high Pnt state. To explore how this network drive cell fate specification in the larval eye disc, we have recombineered fluorescent protein (FP) tags into the *pnt* and *yan* loci and are using confocal microscopy and computational segmentation to examine their expression dynamics in thousands of individual cells at each stage in their development. Using this approach, we expect to achieve an unprecedented level of understanding of the molecular interactions that drive cell fate specification. Our preliminary data challenge several assumptions of the current model. First, we find that Pnt and Yan exhibit similar activation profiles in uncommitted cells and similar degradation patterns in differentiating cells, suggesting that a high Yan/low Pnt state does not define the undifferentiated state nor perduring Pnt expression the differentiated state. Since the *pnt* locus encodes multiple isoforms, we have generated isoform-specific FP fusions to ask how each contributes to overall Pnt dynamics and are using these alleles to test the consequences of isoform-specific knock-downs on cell determination and network dynamics. Our preliminary data suggest more complicated patterns of overlapping and non-overlapping expression in multipotent and differentiating cells than originally anticipated. Finally we are investigating the consequences of manipulating Notch and Egfr signaling levels on Yan and Pnt expression dynamics in progenitors versus differentiating cells. We will discuss the implications and insights that we are uncovering in our quantitative systems-level analyses of Yan-Pnt expression dynamics with respect to current models of retinal cell fate specification.

241C

***In vivo* screen for a novel small molecule inhibitor of PLCγ in *Drosophila*. Chitra Naidu**, Claire Rosenwasser, Todd Rosenberg, Michelle Latino, Justin Thackeray. Biology, Clark University, Worcester, MA.

PLCγ is a key signaling molecule that regulates pathways required for cell proliferation, differentiation and apoptosis. Various studies have reported PLCγ overexpression to be a key factor in transforming primary tumors to metastatic by affecting these very pathways. A PLCγ-specific inhibitor could therefore be an invaluable tool not only for basic research but also for anti-cancer studies.

Small wing (*sl*), the *Drosophila* homolog of *PLCγ*, plays a dual role. It negatively regulates the EGFR pathway controlling photoreceptor and wing vein differentiation while positively regulating the Insulin pathway affecting growth. An *sl* null mutant (sl^{9}/sl^{9}) shows a reduced wing size, ectopic veins and rough eyes as a result of extra photoreceptors in ~60% ommatidia. Our objective is to identify a novel small molecule inhibitor of PLCγ using *Drosophila* as a model system.

In our primary screen, we looked at vein differentiation patterns to identify molecules that alter EGFR signaling. Argos, an inhibitor of EGFR, when overexpressed in wings causes severe loss of venation which is significantly rescued in L3 by a partial loss of SI function. Thus, drug fed MS1096>Aos flies were used as a sensitive model system to detect potential SI inhibition by looking for L3 vein recovery. 37 small molecules showing significant vein recovery were identified among a chemically diverse set of 1596 provided by the NCI. These molecules are being tested further in two secondary screens to confirm EGFR inhibition by looking at photoreceptor differentiation in the eye in various mutant backgrounds. Subsequent experiments will try to determine whether any of the small molecules identified do in fact inhibit SI.

242A

Uncovering a novel interplay between the EGFR and JNK signaling pathways in *Drosophila* dorsal closure. Ze'ev Paroush, Tatyana Shestkin, Sharon Mezuman, Shaked Cohen, Ayala Smotrich, Aharon Helman. Department of Developmental Biology and Cancer Research, IMRIC, Faculty of Medicine, The Hebrew University, Jerusalem, Israel.

Dorsal closure (DC) is a developmental process in which two contralateral epithelial sheets migrate to seal a large embryonic dorsal hole. JNK signaling activity in the dorsal-most leading edge (LE) cells of the epidermis is essential for this process, specifically to induce expression of *decapentaplegic* (*dpp*). Secreted Dpp subsequently triggers cell shape changes in the adjacent ventral epithelial (VE) cells, necessary for the morphogenetic movements and cell migration that occur during DC. We have recently identified an unexpected requirement for the EGFR pathway in DC. In particular, we find that MAPK/Erk is phosphorylated in the lateral epidermis of the embryo in response to EGFR signaling. Notably, EGFR pathway mutants fail to complete DC and, consequently, exhibit dorsal open phenotypes. Genetic and functional analyses indicate that the EGFR pathway acts upstream of JNK signaling, and that it is positively required for the expression of the JNK pathway target gene, *dpp*. Surprisingly, EGFR and JNK pathway activities are evident in distinct cell types: whereas JNK signaling takes place in LE cells, phospho-MAPK/Erk staining is observed in the nearby VE cells. A mechanism at the basis of this novel, non-autonomous interplay between the EGFR and JNK pathways will be presented.

243B

Characterization of Dis3 in *Drosophila melanogaster*. Lindsay Petsch[1], Danielle Bazelak[1], Mark Snee[2], Hemlata Mistry[1], James Skeath[2]. 1) Dept. of Biology, Widener University, Chester, PA; 2) Dept. of Genetics, Washington University School of Medicine, St Louis, MO.

Extracellular stimuli detected by cell surface receptors are relayed to the nucleus via conserved signal transduction pathways, such as the RAS-ERK pathway. Growth factor binding to specific receptor tyrosine kinases at the plasma membrane initiates a signaling cascade to activate ERK, a serine/threonine kinase, via Ras, Raf and MEK intermediaries. The identity and activity of the components of the pathway from Ras to ERK are well understood, but only a few ERK target substrates have been identified and studied. Genetic and biochemical screens conducted in worms and flies have uncovered Dis3 as a putative ERK target. In humans, *dis3* mutations have been identified in patients with multiple myeloma; a lethal neoplasm originating from terminally differentiated antibody-producing B cells. Dis3 protein has 3′ to 5′ ribonuclease activity and functions as a catalytic component of the evolutionarily conserved RNA processing and degradation complex, the exosome. Using polyclonal antibodies against full-length Dis3 protein, we have used western blotting techniques to visualize Dis3 protein expression in wild-type *Drosophila* embryos. To better understand the importance of Dis3 during development, we undertook an EMS-based mutagenesis approach to isolate *dis3* loss of function alleles in *Drosophila*. We have molecularly characterized two *dis3* alleles from this screen.

244C

Src42A Modulates Tumor Invasion and Cell Death via Ben/dUev1a-JNK Signaling in Drosophila. Xianjue Ma, **Yingyao Shao**, Hongyu Zheng, M Li, Wenzhe Li, Lei Xue. Department of Interventional Radiology, Shanghai Key Laboratory of Signaling and Disease Research, Shanghai 10th People's Hospital, School of Life Science and Technology, Tongji University, Shanghai 200092, China.

Loss of the cell polarity gene could cooperate with oncogenic Ras to drive tumor growth and invasion, which critically depends on the JNK signaling pathway in Drosophila. By performing a genetic screen, we have identified Src42A, the ortholog of mammalian Src, as a key modulator of both RasV12/lgl-/- triggered tumor metastasis and loss of cell polarity gene induced cell invasion. Our genetic evidence further established Bendless (Ben)/dUev1a E2 complex as an essential regulator downstream of Src42A. Reducing Ben/dUev1a activity suppressed Src42A overexpression induced cell invasion and cell death, while ectopic expression of Ben/dUev1a restored src42A loss thorax cleft defect. Furthermore, we showed Ben/dUev1a expression cells could migrate invasively in wing disc epithelia along with

increased MMP1 secretion. Moreover, when RasV12 is co-expressed, Ben/dUev1a expressing cells promoted tumor overgrowth and caused invasion into the ventral nerve cord. Our data provides new mechanistic insights into in vivo role of Src42A in regulating tumor progression and highlights the importance of Src42A- Ben/dUev1a- JNK signaling in regulating tumor invasion and cell death in Drosophila.

245A

Investigation of novel epidermal growth factor receptor signaling target genes implicated in *Drosophila* development. Connor Zale, Sean Thomas, Jeffrey Perluke, Michael Warkala, Lisa Kadlec. Dept. of Biology, Wilkes University, Wilkes-Barre, PA.

Signaling by the *Drosophila* epidermal growth factor receptor (Egfr) plays an important role in many aspects of development, including oogenesis, embryogenesis and proper development of both the wing and the eye. The Egfr pathway is utilized, for example, in the establishment of the body axes during oogenesis, in vein tissue specification in the wing, and in photoreceptor cell differentiation in the eye. Microarray screens by our lab and others have previously been used to identify novel downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression using RNA isolated from fly ovaries in which the activity of the Egfr-pathway was reduced (grk-HK36 mutant), normal (OreR), or constitutively active (CY2/λtop transgenic). We have been employing a variety of cell biological and genetic approaches to investigate the expression, biological function, and mechanism of action of our putative targets. Target genes of interest include a number of genes whose function is currently unknown (including CG13299, CG11381, CG13083 and CG14309). RT-PCR has confirmed the up-regulation of a subset of genes originally identified by microarray. Several putative targets have been shown to exhibit developmentally regulated expression in the ovary, and in some cases this expression has been shown to be altered in response to changes in levels of Egfr signaling. Screening for biological function using targeted knockdown via UAS-RNAi suggests roles for several targets of unknown function in eggshell production and/or integrity, wing morphogenesis, or both. Neutral red uptake assays and vitelline membrane-targeted RNAi suggest a role for vitelline membrane defects in compromised eggshells. We have used *in situ* hybridization and RT-PCR to investigate target gene expression in wing imaginal discs. Additionally, we are utilizing the UAS-RNAi system to attempt to identify candidate target genes which may play roles in the development of the eye.

246B

Invadolysin: a novel metalloprotease plays an important role in metabolism. Kanishk Abhinav, Margarete M.S Heck. University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK.

Invadolysin is a conserved and essential metalloprotease that localises to lipid droplets, the intracellular lipid storage organelle. A functional role in lipid metabolism is suggested by the observation that*invadolysin* mutant larvae accumulate significantly lower amounts of triglycerides (Cobbe *et al*. 2009). We previously showed that invadolysin is important for cell migration and cell division (McHugh *et al*.2004), physically interacts with ATP synthase subunits and is required for mitochondrial function (Di Cara *et al*. 2013). Morpholino knockdown of invadolysin in *D. rerio* demonstrated that invadolysin is also required for cell migration in zebrafish, as defects in the deposition of neuromast clusters, melanophore distribution and angiogenesis were observed (Vass and Heck 2013). Our recent studies have demonstrated that insulin signalling is impaired in *Drosophila invadolysin* mutant larvae, and that animals have low glycogen levels and are unable to accumulate triglycerides on a high sugar diet. We are therefore investigating the role of invadolysin in metabolism and its catalytic activity. Invadolysin has a conserved metalloprotease motif and a potential lipase motif. We have generated transgenic*Drosophila* lines that overexpress either wild type, protease- or lipase-dead versions of invadolysin. We are using these lines to analyse the roles these motifs in the activity of invadolysin, and will discuss the phenotypic consequences of expressing aberrant forms of invadolysin.

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247C

Cdk8 and Sgg control the intensity and range of the BMP-activity gradient in developing *Drosophila* tissues. Abigail Aleman, Hugo Urrutia, Edward Eivers. California State University Los Angeles, Los Angeles , CA.

Embryonic patterning is established by growth factor gradients within specific tissue fields, and cells must interpret these gradients to accomplish precise developmental management. An important developmental pathway is the bone morphogenetic protein (BMP) pathway; which instructs embryonic cells to divide, differentiate or die, while aberrant BMP signaling has been implicated in an array of developmental disorders. The BMP signal is propagated intracellularly through C-terminal phosphorylation of its transcription factor Mad, which forms an activity gradient in developing tissues. Here we are interested in how Mad linker phosphorylations control the intensity and duration of the BMP signal in blastoderm embryos and wing imaginal discs. Under investigation are Mad linker serines 204, 208, and 212. Specifically, we want to understand how the combined activity of two kinases, Cyclin dependent kinase 8 (Cdk8) and Shaggy (Sgg) generates a mirroring linker phosphorylation gradient to control peak intensity and range of the BMP activity gradient. We propose that linker hyper phosphorylation is a cellular mechanism essential for regulating the BMP activated Mad (pMad) gradient in developing tissues. .

248A

Epithelia patterning and body plan mapping in the *Drosophila* egg chamber. Alexis L. Braun, Bhavna Chanana, Isabel M. Palacios. Zoology, University of Cambridge, Cambridge, United Kingdom.

Cell fate specification in response to patterning cues to form specialized tissues is a fundamental process in development. A clear example of this is the patterning of the follicular epithelium (FE) surrounding the female germline of *Drosophila melanogaster*, the correct patterning of this tissue is crucial for establishing the axes underpinning the embryonic body plan. The different groups of cells forming the FE start from one type of cell, the follicle stem cell, and go through successive waves of differentiation to make up the different types of cells in the FE. A group of defined cells, known as posterior follicle cells (PFCs), are specified by a signal from the oocyte to take the posterior fate. These PFCs then signal back to the oocyte by an unknown mechanism inducing the reorganization of the cytoskeleton, thus setting the anteroposterior and dorsalventral axes. To understand the process whereby PFCs adopt their fate and induce asymmetry in the oocyte, we designed a cell-specific expression profiling screen comparing the profile of PFCs to that of the remaining body of follicle cells. From this, we identified a number of candidate genes, including membrane proteins, transcription factors, and G-protein receptors, as well as many that were previously characterized in the FE. We then employed three overlapping functional screens - mutant allele, overlapping deficiency, and RNAi - to sift through all of the candidates with increased expression in the PFCs and identify genes essential for oocyte polarization. By these means we identified nine candidate genes that have an interesting phenotype. Most of these have phenotypes relating to a loss of cell fate, with substantial disruption to the tissue architecture of the epithelium. One of these genes, an undescribed transmembrane protein, shows oocyte polarizing defects when knocked down by RNAi, with no accompanying epithelial phenotype. Because oocyte repolarization is disrupted, while follicle cell fate appears to be unaffected, this gene may be involved in the crucial step of signaling back to the oocyte. In order to characterize the function we used the CRISPR/Cas9 system to create a mutant and preliminary results support the findings from the knock down.

249B

Spatiotemporal Patterning of Polyamine Metabolism during *Drosophila* Development. Miranda Burnette, Gabrielle Dohmen, Galvin Loughran, Steven Penny, Jeremiah Zartman. Chemical Engineering, University of Notre Dame, Notre Dame, IN.

Polyamines (PAs) are ubiquitous organic molecules that influence many cellular processes such as gene regulation, signal transduction, cell growth, and cell proliferation. Previous research has demonstrated that the metabolic regulation of PAs plays important roles in various diseases including cancer, Multiple Sclerosis, and Parkinson's disease. However, despite their multifaceted nature, the regulation and mechanistic roles of PAs remain unclear. By performing bioinformatics analysis on previously published gene expression data across *Drosophila* development, we discovered that PA regulatory genes cluster with the enzymatic flow of PA metabolism. This led to the identification of striking dynamic spatio-temporal patterns of PA accumulation during specific aspects of embryonic and larval development. Leveraging the genetic tools of *Drosophila*, we have characterized PA pathway functionality in a variety of biological processes, including retinal differentiation in the larval eye imaginal disc, tubulogenesis in the developing embryo, and tumor initiation and metastasis in a larval cancer model. Due to the high level of conservation of the PA pathway in eukaryotes, a thorough understanding of PA regulation and functionality can provide new insight at the interface between metabolism and growth during organogenesis as well as for developing new strategies targeting tumor growth and metastasis.

250C

Exploring potential downstream targets of Atrophin in Fat/Dachsous PCP signaling using DamID. C. Kuok[1,2], A. Soltyk[3], M. Fanto[4], T. Westwood[3], H. McNeill[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Department of Cell and Systems Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada; 4) MRC Centre for Developmental Neurobiology, King's College London, London, UK.

Planar cell Polarity (PCP) is the coordinated polarity of a group of cells within the plane of tissue, perpendicular to the apical-basal axis. In *Drosophila*, PCP pattern is evident in the orientation of wing hairs, wing ridges and photoreceptor clusters in the eye. Previous studies have shown that Atrohpin (also called *grunge*) is involved in Fat/Dachsous (Ft/Ds) PCP signaling. As Atrohpin is a conserved transcriptional corepressor, we hypothesize that Atrohpin mediates Ft/Ds PCP signaling through transcription regulation. To identify the potential downstream targets of Atrophin, we utilized DNA adenine methyltransferase identification (DamID), a DNA binding site mapping technique, using eye-brain complexes. To study the potential roles of these genes in PCP, we plan to examine wings and eyes using RNAi and mutant flies.

251A

Poly: a novel protein with an essential role in metabolism and Type 2 Diabetes. Ioanna Panagakou, Margarete M.S Heck. University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK.

Poly is a conserved and essential protein with sequence homology to Elp6, a member of the Elongator complex (Bolukbasi *et al.,* 2012), which exhibits acetyl transferase activity towards multiple substrates (Svejstrup, 2007). Poly binds to the Insulin Receptor and mutant larvae exhibit increased autophagy in the larval fat body and altered metabolism (Bolukbasi *et al.,* 2012). The predicted molecular weight of Poly is 28 kDa (Poly^{28}). Our recent work has revealed two novel forms, which may arise through post-translational modification, and show differential expression during development and in response to different diets. We have been analysing Poly in a *D. melanogaster* type 2 diabetes model (Musselman *et al.,* 2011). Wild type animals fed on high sucrose until the third instar larval stage developed severe hyperglycaemia and insulin resistance. Both *poly* mutant and diabetic larvae had similar phenotypes such as

prolonged third instar larval stage and smaller body size. TAG:protein levels of those larvae are also significantly decreased, whereas autophagy is increased. All the above suggest an important role for Poly in metabolism and the InR/TOR pathway. To complement our findings, we have generated two *Drosophila* transgenic strains tagged with tRFP either at the N' or the C' terminus of Poly. The manipulation of these transgenic flies is facilitating the examination of *in vivo*of the role of Poly in metabolism.

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252B

The role of G protein o alpha subunit in the eye development and aging in *Drosophila*. Silvia Plascencia Oliveros[1,2], Marlène Cassar[1], Doris Kretzschmar[1]. 1) Oregon Institute of Occupational Health Sciences, OHSU, Portland, OR; 2) Biology, University of Portland, Portland, OR.

While Alzheimer's Disease is typically associated with amyloid precursor protein (APP) and Aβ plaque formation, we wish to investigate an alternate, neuroprotective pathway of APPL (amyloid precursor protein-like) in the *Drosophila* model. Previous studies from our laboratory show that APPL can be protective in selected neurodegenerative background. Our preliminary results suggest that downstream effects of active Goα subunit are involved in a neuroprotective pathway of APP signaling via ion channel modulation. To investigate the role of the Goα protein, we focused on determining neurodevelopmental and neurodegenerative consequences of Goα misregulation through the*Drosophila* eye model. Taking advantage of the Gal4/UAS system, we decided to overexpress or inhibit Goα throughout development and adulthood by performing several types of assays: 1- Immunohistochemistry to assess photoreceptor patterns with confocal microscopy using an anti-chaoptin antibody, 2- Whole brain paraffin sections to assess brain structure integrity, and 3- Fast phototaxis assay to assess visual function integrity. All analyses were performed on young (3 days) and older flies (14 and 30 days) kept at 25°C. Our preliminary data suggests that Goα overexpression only induced severe behavioral defects compared to controls in young flies whereas Goα inhibition add little or no effect. This effect did not worsen with age suggesting a defect in neurodevelopment rather than in the aging process. We also observed severe eye neurodegeneration in young flies overexpressing Goα, but have not yet associated these defects with photoreceptor defects. Our preliminary study suggests a strong involvement of Goα in photoreceptor development and maintenance.

253C

The Abelson kinase prevents photoreceptors from adopting pigment cell fate. Nicelio Sanchez-Luege[1,2], Ilaria Rebay[3]. 1) Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL; 2) Medical Scientist Training Program, University of Chicago, Chicago, IL; 3) Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

The advent of induced pluripotent stem cells suggests that terminally differentiated cells can retain fate plasticity throughout development, but the mechanisms that regulate this plasticity *in vivo* remain poorly understood. Our study will address how neuronal fate is stabilized following specification and the extent to which cells can deviate from this fate when maintenance mechanisms are disrupted. Our lab previously showed that loss of the Abelson kinase (Abl) causes photoreceptors in the developing eye to lose expression of neuronal markers during mid/late pupal stages, a phenotype that suggests that retinal cell fates are not irreversibly determined even two days post-specification. What is the ultimate fate of *abl* photoreceptors? We present evidence that some *abl* photoreceptors adopt the fate of interommatidial pigment (IOP) cells. We find that retinal *abl* clones induced by mitotic recombination show an increased number of cells expressing known IOP markers, including the IOP-specific enhancer trap LL54-Gal4. Furthermore, we can find *abl* photoreceptors with high co-expression of Elav, a marker of neuronal fate, and LL54 as early as 24 hours following pupal formation, suggesting the existence of a transition state in which genetic programs corresponding to both fates are simultaneously active. We are performing a lineage trace of *abl* photoreceptors to explore the extent to which these cells ultimately mimic IOP structure and function. In other words, is there a bona fide fate transition, or are the cells only exhibiting aberrant genetic programs? We also notice that dual-marker expressing cells localize near clonal borders, raising the possibility that the transition state is more pronounced in these areas due to non-autonomous signals sent from nearby wildtype cells to promote maintenance of neuronal fate. We are identifying these maintenance signals to better understand the molecular mechanism of *in vivo* cell fate maintenance.

254A

The phenotypic effects associated with loss of PKN function are manifest upon perturbation of cell death in *Drosophila melanogaster*. Georgette Sass, Jessica Schoenherr. Biology, Grand Valley State University, Allendale, MI.

The *Drosophila* homolog of the mammalian PKN family kinases is required during the embryonic process of dorsal closure and has been identified as an effector of Rho1, a member of the Ras GTPase superfamily. The loss-of-function, dorsal closure phenotype associated with the mutant allele pkn^{06736} is relatively mild (~10% of embryos with the phenotype) and may reflect functional redundancy with components of the Jun-terminal kinase (JNK) pathway that are also required for dorsal closure in *Drosophila*. We have investigated post-embryonic phenotypic consequences associated with loss of PKN function using this same mutant allele and via ectopic expression of *pkn* RNAi. Low levels of lethality are seen throughout early stages of development in pkn^{06736} homozygotes, however all are individuals are dead by the end of the third instar with accompanying defects in formation of imaginal discs. In contrast, ectopic expression of PKN RNAi does not result in a mutant phenotype associated with loss of PKN function in any of the adult

structures examined (i.e. using ovary, wing and eye GAL4 drivers). However, overexpression of the death-inducing gene, *head involution defective* (Hid) in the eye while also removing PKN using the eye specific promoter (*GMR-Pkn RNAi*) results in a larger eye size suggesting that PKN loss leads to cell survival. Cell survival does not seem to be impacted by *Erk*'s inactivation of Hid since the same effect is also seen when a mutant of *hid* (*hid*^{ala5}) is used. These results suggest that PKN may promote cell death and that its function is independent of the Ras/MAPK survival pathway. We find that phenotypic effects of PKN loss of function in the adult are not seen in the absence of ectopic perturbations of cell death. This indicates that even though PKN is required earlier for larval development, PKN function in adult tissues is only important if the cell death/survival pathways are altered.

255B

Coordinated regulation of tissue remodeling and insulin signaling during *Drosophila* metamorphosis. Craig Woodard, Giulia Notarangelo, Bezawit Woldemeskel, Hanna Cho, Yeonhee You, Efthymia Papalexi. Department of Biological Sciences, Mount Holyoke College, South Hadley, MA.

Animal development requires the precise coordination of many diverse events. As development progresses, animals must destroy certain tissues as they build or remodel others. It is important to understand the mechanisms by which animals precisely control the timing of morphogenesis, cell death, tissue remodeling, and nutrient release. Holometabolous insects undergo complete metamorphosis, during which they build an adult body, while eliminating obsolete larval tissues by programmed cell death. In contrast, tissues required for further development are spared from total destruction and remodeled to meet the needs of the adult fly. The *Drosophila* larval fat body is an organ that escapes destruction during metamorphosis and is instead remodeled, breaking up into individual cells that are distributed throughout the developing adult body. The fat body must release nutrients in a controlled manner during metamorphosis, which is a time when the animal is not feeding for several days. We have determined that modulation of 20-hydroxyecdysone (20E) signaling by the nuclear hormone receptor ßFTZ-F1 induces fat body remodeling and expression of *Matrix metalloproteinase 2* (*Mmp2*) in the fat body. Furthermore, *Mmp2* is necessary and sufficient to induce fat body remodeling. However, the control of insulin signaling and nutrient release by the fat body during metamorphosis has not been examined in detail. Our recent findings suggest that 20E signaling and ßFTZ-F1 regulate insulin signaling in the larval fat body during metamorphosis. We are testing the hypothesis that 20E signaling and ßFTZ-F1 regulate tissue remodeling and insulin signaling in the fat body via a common mechanism that involves the action of Mmp2. Coordinated regulation of tissue remodeling and insulin signaling in the fat body would ensure that nutrients needed by the developing fly are released by the organ with proper spatial and temporal control.

256C

Targeted mutagenesis and functional analysis of *Drosophila* adipokinetic hormone gene. M. Zurovec[1], S. Sajwan[1], R. Sidorov[1], Y. Takasu[2], T. Staskova[1], A. Zaloudikova[1], D. Kodrik[1]. 1) Biology Centre Czech Acad. Sci., Ceske Budejovice, Czech Republic; 2) 2National Institute of Agrobiological Sciences, 1-2 Owashi, Tsukuba, Ibaraki 305-8634, Japan.

AKHs are small insect peptides (8-10 amino acid residuess) that regulate metabolic responses to stress by stimulating catabolic reactions and mobilizing energy stores. We employed targeted mutagenesis using TALENs and isolated *DmelAkh*^{1} mutant carrying a small deletion in the ORF. Our results show that *DmelAkh*^{1} mutants are fully viable, have significantly lower level of hemolymph glycides including trehalose, are less sensitive to starvation, and exhibit lower level of total metabolism when compared to control flies. Similar phenotypes were observed earlier in flies carrying the targeted ablation of AKH expressing neurons. The *DmelAkh*^{1} phenotypes can be rescued by ectopic expression of *DmelAkh*. Taken together, our data show that we have generated a full null mutation of *DmelAkh* gene. We are currently investigating the role of AKH in various stress conditions.

257A

Multiple roles for the Netrin receptor, Frazzled, in EMT. S. Golenkina[1], R. Manhire-Heath[1], R. Saint[2], M. J. Murray[1]. 1) The University of Melbourne, Australia; 2) The University of Adelaide, South Australia.

Epithelial Mesenchymal Transitions are important in cancer metastasis and development. We are studying the EMT-like event occuring during wing eversion, whereby peripodial cells degrade the basement membrane (BM) via MMPs, lose apico-basal polarity, delocalize ECadherin and extend FActin rich protrusions during invasion and migration over the epidermis. Eversion failure leads to disrupted wing/thorax formation. In an RNAi screen we identified the chemoattractant *netrinA*. *net*ARNAi suppressed ECad dissociation, but not FActin reorganization, JNK activation, or BM breakdown. Several lines of evidence show NetA promotes EMT by repressing Frazzled (Fra): 1) *fra*RNAi accelerates EMT; 2) *net*ARNAi increases Fra levels; 3) *net*ARNAi phenotypes are suppressed by reducing *fra*levels; 4) excess Fra blocks eversion; 5) Fra acts through Moesin phosphorylation; 6) *moe*RNAi rescues *net*ARNAi phenotypes*. To determine the downstream pathways we are studying the ability of different *fra* deletion transgenes to produce adult and cellular phenotypes. FraFL (full length Fra) and FraΔP2 (lacking the cytoplasmic P2 domain) caused eversion failure, but FraΔP1ΔP2 and FraΔP3 had no effect. Thus, inhibition of eversion needs the P1 and P3 domains. Mosaic expression of FraFL in wing discs caused: 1) disrupted apico-basal localisation of pMoe and ECad; 2) a widening of the basal side of disc proper cells, suggesting repression of Rho1 contractility; 3) extensive FActin/microtubule rich protrusions, presumably due to the known Fra axon guidance pathway. No transgene was able to disrupt Moe/Ecad localisation but both FraΔP1P2 and FraΔP2 generated extensive protrusions. Since FraΔP1P2 could cause protrusions, but not block eversion, the eversion pathway is separable from the motility pathway. FraΔP3 induced fewer and smaller protrusions, consistent with the known requirement of the P3 domain in axon guidance. FraΔP2 produced basal relaxation, suggesting that this phenotype may be related to eversion failure. We are now testing the requirement of potential downstream pathway components, such as putative Moesin kinases (e.g. SLIK, Srk, PI3K, *etc*.), RhoGAP Conundrum and Myosin, for the Fra phenotypes. *Manhire-Heath *et al*.,

2013, *Nat. Comms*. 4, 2790.

258B

Functional understanding of Ral signaling in Drosophila melanogaster. Helene Knaevelsrud[1], Marc Therrien[1,2]. 1) IRIC, Universite de Montreal, Montreal, Quebec, Canada; 2) Département de pathologie et de biologie cellulaire, Université de Montréal.

The small GTPase Ral regulates important membrane trafficking events, including exocytosis, endocytosis and autophagy. In mammalian cells Ral is activated downstream of active Ras, which directly interacts with RalGEFs. Over the recent years it has become clear that Ral plays an important role in signal transduction leading to cancer formation and metastasis, both in Ras-dependent and independent manners. Yet, little is known about the Ral signaling network. To understand more about Ral signaling in development and carcinogenesis, we have characterized the effect of expressing constitutively active RalG20V and dominant negative RalS25N in various fly tissues, including wings, notum and eyes. Furthermore, we have analyzed the dependence of these phenotypes on known and predicted regulators and effectors of *Drosophila* Ral. Upon RalG20V expression we observed misorientation of bristles in the notum, wing vein defects and misaligned ommatidia. Conversely, expression of RalS25N led to loss of notum bristles, small and malformed wings and mild eye roughening. We further characterized the RalS25N-induced balding of the notum and found it to be suppressed by coexpression of the RalGEF Rgl or wild-type Ral, by introducing mutations in the exocyst component Sec5 - a known Ral effector - or by reducing JNK signaling – a known downstream pathway negatively regulated by Ral signaling. In conclusion, we present fly models for Ral signaling amenable to genetic screening that will allow us to identify new components of the Ral signaling network. .

259C

Nanobody-mediated morphogen trapping - Growth and patterning in the absence of a Dpp gradient. S. Harmansa, M. Affolter, E. Caussinus. Biozentrum, Universität Basel, Basel, Switzerland.

Morphogens are secreted signaling molecules forming concentration gradients and controlling organ patterning and growth. Drosophila Decapentaplegic (Dpp) is one of the best studied morphogens, but it is still unclear how its concentration gradient controls patterning and growth of the *Drosophila* wing imaginal disc. Here we use a novel nanobody-based tool, Morpho-trap, to block Dpp gradient formation and study the effect of the loss of the gradient on patterning and growth. We find that induction of Dpp target genes, and hence patterning, directly depends on the spreading of Dpp. Furthermore, we show that the Dpp gradient is crucial for growth and size control of the *medial* wing disc region (where Dpp levels are high). However, we find that the Dpp gradient is not necessary for proliferation and size control of the *lateral* region of the wing disc (where Dpp levels are low). This data challenges previously published models, in which growth control depends solely on the signaling dynamics of Dpp. The actual size control mechanism of the wing imaginal disc appears to be more complex since it involves Dpp-independent control mechanisms for the lateral cells.

260A

Dissecting signaling crosstalk in the midgut with dynamic transcriptional reporters. David P Doupé[1], Benjamin E Housden[1], Li He[1], Richard Binari[1], Norbert Perrimon[1,2]. 1) Dept of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, Boston, MA.

How intercellular signaling determines cell fate or behavior is a critical question in cell and developmental biology. Although a great deal is known about which pathways are involved in many processes, it often unclear how they interact. The Drosophila midgut is an excellent model for the role of signaling crosstalk in cell fate. In normal homeostasis intestinal stem cells (ISCs) self-renew and generate enteroblasts, which differentiate into absorptive or secretory cells. In response to damage, turnover is dramatically accelerated to repair the tissue. Many pathways, such as Notch, cytokine JAK/STAT, EGFR, JNK and Hippo, have been implicated in regulation of ISCs and epistasis studies have identified many points of signal crosstalk. However, most studies have been conducted over a timescale of days while cellular responses to signaling and pathway interactions may occur on a timescale of minutes. A systematic understanding of how these pathways interact in space and time is therefore lacking. Effects on cell fate and induction of secondary pathway responses are often mediated at the transcriptional level, for example one pathway inducing another's ligand. We are therefore developing novel transcriptional reporters using translation-enhanced super-folder GFP (sfGFP). Transgenic reporters using pathway responsive promoter elements give a read-out of activity but may not accurately reflect the expression of endogenous targets and cannot reveal the expression of upstream components such as ligands. Using CRISPR/Cas9 with homologous donors we are targeting sfGFP to the endogenous translational start sites of key signaling genes such as ligands to allow their expression to be characterized at high spatiotemporal resolution. Using a combination of fixed samples and live imaging this is allowing us to analyze the relationships between pathways in ISC regulation.

261B

Computational modeling of transient EGFR signaling in the early embryo. Yogesh Goyal[1,2], Bomyi Lim[1,2], Andreas Raue[3], Birgit Schoeberl[3], Stanislav Shvartsman[1,2]. 1) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Merrimack Pharmaceuticals, 1 Kendall Square, Cambridge, MA.

The epidermal growth factor receptor (EGFR) system has been at the forefront of studies of receptor-mediated cell responses, providing a paradigm for the quantitative studies of signal transduction. A lion's share of the work has been done with cells in culture, where the numbers of ligand-receptor complexes needed to trigger a specific event and the temporal progression of cell responses can

be studied relatively straightforwardly. For instance, ligand production and receptor expression can be quantified by analyzing the extracellular medium conditioned by cells and studies with labelled ligands. In contrast, endogenous signals in developing tissues are more challenging to quantify, because even some of the most basic assays available for studies with cultured cells do not have analogs in the context of whole tissues. Here, we use the early *Drosophila* embryo as an experimental system for establishing quantitative models of EGFR signaling *in vivo*. We focus on the first round of EGFR signaling during*Drosophila* embryogenesis, when a spatiotemporal pulse of EGFR activation gives rise to the expression of *ind*, a gene essential for patterning of the nerve cord. Based on our data from high-throughput imaging of *Drosophila* embryos in microfluidic devices, we formulated a five-variable dynamic model that accounts for the spatiotemporal pattern of EGFR activation. We used a deterministic optimization algorithm to estimate about a dozen of model parameters and then used these estimates to explore the systems-level properties of tissue patterning by a pulse of EGFR signaling. We will present our results on the identifiability of model parameters and experimental tests of model predictions.

262C

The Hippo Signaling Pathway Interactome. Young Kwon[1], Arunachalam Vinayagam[1], Xiaoyun Sun[3], Noah Dephoure[4], Steven Gygi[4], Pengyu Hong[3], Norbert Perrimon[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA; 3) Department of Computer Science, Volen Center for Complex Systems, Brandeis University, Waltham, MA; 4) Department of Cell Biology, Harvard Medical School, Boston, MA 02115.

The evolutionary conserved Hippo signaling pathway plays critical roles in the control of metazoan organ growth by regulating both cell proliferation and apoptosis. While a number of components have been identified in recent years, our knowledge of the composition and structure of this pathway is still incomplete. Using existing pathway components as baits, we generated by Mass Spectrometry a high-confidence *Drosophila* Hippo protein-protein interaction network (Hippo-PPIN) consisting of 204 nodes and 143 edges. Analysis of protein sub-complexes, using a newly developed method COMPLEAT, revealed that the Hippo-PPIN is enriched for complexes involved in endocytosis/vesicle trafficking, cytoskeleton organization, nucleo-cytoplasmic transport and spindle organization. Hippo-PPIN contains many potential new components of Hippo signaling. Here, we selected for further characterization a new member of the alpha-arrestin family, Leash, and show that it binds and promotes degradation of the transcriptional co-activator Yorkie (Yki) through the lysosomal pathway. As the Hippo pathway is evolutionary conserved with many components implicated in either tumor suppression or progression, further characterization of Hippo-PPIN will contribute to our understanding of this network in both normal growth and cancer.

263A

***microRNA-9a* canalizes myotendinous junction assembly via targeting of muscle-specific genes. Andriy Yatsenko**, Halyna Shcherbata. Max Planck Research Group of Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077, Goettingen, Germany.

Precise timing, expression pattern and amounts of protein expression is key for organism development and tissue maintenance. microRNAs modulate gene expression in response to perpetually changing external and internal conditions. In our study, we found that Drosophila *miR-9a* is involved in canalization of myotendinous junction (MTJ) assembly. *miR-9a* deficiency affects embryonic survival, a phenotype that can be rescued by specific expression of this miRNA in tendon cells. Interestingly, the survival of *miR-9a* mutants depends on the speed of embryonic development that reciprocally correlates with transcriptional noise. *miR-9a* is expressed in epidermally-derived tendon cells, while many *miR-9a* predicted targets are essential muscle genes which are misregulated due to *miR-9a* loss and gain of function. Moreover, exogenous expression of *miR-9a* in mesoderm completely abolishes muscle formation. Therefore, we put forward a hypothesis that *miR-9a* adjusts tendon cell differentiation by preventing misexpression of muscle genes resulting from stress or aberrant transcription. To prove this hypothesis, we misexpressed putative *miR-9a* targets in tendon cells and found that ectopic htl (heartless), wit (wishful thinking) and Dg (Dystroglycan) in tendons cause muscle attachment and embryonic lethality phentotypes similar to those found in *miR-9a* mutants. In particular, we found that the muscular dystrophy-associated ECM receptor, Dg is regulated posttranscriptionally via *miR-9a*. During the early embryonic stages, Dg is present in all epidermal cells; however, for proper assembly of muscle attachment sites it is essential that Dg is eliminated from epidermally-derived tendon cells, with *miR-9a* modulating the precision of this expression. When Dg is misexpressed in tendon cells, the composition of the tendon matrix is affected, resulting in aberrant muscle attachments and embryonic death. Based on obtained data, we propose that *miR-9a* acts as a guardian to prevent aberrant muscle gene expression in the epidermal tendon precursor cells.

264B

Proteomic discovery of a novel mechanism linking calcium signaling and p38MAPK pathway activation. Vladimir Belozerov, Lisa Shim, April Kong, Reza Amirzadeh, Arthur Hilliker, Spencer Mukai, John McDermott. Department of Biology, York University, Toronto, ON, Canada.

The p38 MAPK pathway is a key evolutionarily conserved mediator of an organism's response to stressful environmental stimuli. In mammals four p38 kinases form a robust signaling module. In *Drosophila* the p38 MAPK family consists of two highly homologous kinases, Mpk2 and p38b, and the third putative kinase p38c. Among these, p38b plays a central role in stress-induced signal transduction. Although the canonical three-tier p38 activation cascade is well understood, less is known about the specific factors sensitizing the core cascade to inputs from other signaling pathways. To uncover these putative pathway interactions, we examined the *in vivo* interactome of p38b. We used an optimized single-step affinity purification procedure followed by gel-free LC-MS/MS analysis to detect both stable, and more transient, lower-affinity interactions. One of the prominent interactors is a highly-conserved

protein Caliban (Clbn). Proteomic mapping of the p38b-Clbn interaction showed that only a specific proteolytic fragment of Clbn, and not the full-length protein, binds to p38b. We found that this fragment is robustly produced by calpain-mediated cleavage that is activated by intracellular calcium in cultured cells and *in vivo*. We show that in the context of the p38b-Clbn complex the Clbn fragment acts as a scaffold to recruit the upstream activating kinase licorne to the complex thereby potentiating the MAPK signal transduction cascade. Importantly, this mechanism of pathway potentiation appears to operate in all tested p38b activation assays. These biochemical findings are further supported by the observed genetic interactions between *p38b* and the alleles of *Clbn* as measured by p38b activation and downstream signaling. We propose a model of cross-talk between the p38MAPK and calcium signaling pathways with Clbn serving as key mediator. .

265C

Mitochondria are maintained by local fusion and mitophagy at neuromuscular junction synapses. Ryan Insolera[1], Ruohan Wang[1], Pushpanjali Soppina[1], Grace Kim[1], Eric Robertson[1], Yih-Woei Fridell[2], Catherine Collins[1]. 1) MCDB Department, University of Michigan, Ann Arbor, MI; 2) University of Connecticut, Allied Health Sciences, Storrs, CT.

Mitochondria are neither stationary nor solitary: they move, fuse with one another, and divide (fission). While it is clear that these dynamics are critical for neuronal function and survival, most studies of mitochondrial fusion, fission and turnover have been restricted to non-neuronal cells. A few studies have examined mitochondrial dynamics in cultured neurons, however the regulation of mitochondria *in vivo,* where axons are myelinated and form synapses that are regulated, is not well modeled in cultured cells. We propose that neurons use *local* mechanisms to regulate mitochondrial transport, dynamics (fission and fusion) and destruction in response to changing needs.

Our approach to this goal takes advantage of powerful genetic and live imaging techniques in *Drosophila* larvae. We have developed a photoconversion and time lapse imaging assay that allows us to track the transport, behavior and turnover of individual photoconverted mitochondria within and between different regions of individual motoneurons in live animals. Our preliminary data indicate that mitochondria at NMJ synapses are remarkably stable and stationary, however their composition is dynamic: they are supported by continual fusion of new material that originates from the cell body, and removal of old material which depends upon autophagy machinery.

266A

Chromatic Assembly Factor Complex Component p180 is a Potential Component of the Hippo Pathway. William B. Yee, Patrick Delaney, Pamela Vanderzalm, Richard Fehon. Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The conserved Hippo signaling pathway controls tissue growth in a variety of physiological contexts. One of the upstream regulators of the Hippo pathway is a FERM domain containing protein Merlin (Mer). To understand the function of Mer, we conducted a large-scale yeast two-hybrid screen and have found that the chromatin assembly factor-1 (CAF-1) complex component p180 interacts with Mer. Interestingly, Mer is transcriptionally upregulated in p180-deficient cells, in a manner that is dependent upon Yorkie (Yki), the transcriptional co-activator that operates downstream of the Hippo pathway. Furthermore, we showed that overexpression of p180 attenuates the activity of a luciferase-based Hippo pathway reporter, but does not appear to affect expression of transcriptional reporters that are Yki-independent. In addition, heterozygosity for a p180 null allele suppresses the lethality of Yki mutant clones in wing and eye imaginal discs. These data suggest that p180 acts as a transcriptional repressor of Hippo pathway targets and lead us to propose a novel model in which Mer interacts with p180 to regulate Yki's function. Currently, to further elucidate the molecular mechanism of p180's function in the Hippo pathway, we are investigating interactions between p180 and other downstream members of the pathway to ask if p180 participates in the Yki-Sd transcriptional complex, and further investigating the functional significance of the p180-Merlin interaction.

267B

In vivo RNAi knockdown of the *Drosophila* N-acetyltransferase NatA causes cell death. Joseph Ahlander[1], Kelsey Dozier[2], Ethan Hayman[2], Pamela Martin[1], Amanda Scholes[2]. 1) Department of Natural Sciences, Northeastern State University, Tahlequah, OK; 2) Department of Biological Sciences, Univeristy of Arkansas, Fayetteville, AR.

Nα-acetylation is a common protein modification in eukaryotic cells. The major Nα-acetyl-transferase complex, NatA, is composed of the catalytic subunit Ard1 and an auxiliary subunit Nat1, both of which are conserved in higher eukaryotes. NatA plays a role in development and cancer, but its role in these processes is not fully understood. Our goal is to use *Drosophila* to investigate the cellular functions of Ard1 in order to understand its role in disease. We have discoverd that RNAi knockdown of the *Drosphila* ortholog of Ard1 (dArd1) causes lethality and cell death. Knockdown of dArd1 in during eye development results in a small adult eye, which was suppressed by genetic inhibition of apoptosis. Co-expression of a wild-type dArd1 transgene rescued the small eye phenotype, whereas the phenotype was not rescued by expression of a catalytic mutant or a wild-type dArd1 with a nuclear localization signal. These results suggest that the primary function of dArd1 is acetylation of proteins in the cytoplasm. This genetic model will allow us to elucidate the cellular pathways through which NatA functions.

268C

dCAF1 is required for the cell death sensitivity of *rbf1* mutant cells. Heather Collins, Nam-Sung Moon. McGill University, Montreal, QC, Canada.

The loss of the tumor suppressor Retinoblastoma (Rb) leads to cell proliferation and differentiation defects but also increased

apoptosis in particular contexts. The homologue in flies, *rbf1,* also has these properties when absent and offers a more simple system for studying the role of Rb in cell death. Previously we noted the involvement of the histone chaperone complex dCAF1 in the cell death sensitivity of*rbf1* mutant eye imaginal discs. Here we show that this interaction extends to other developmental contexts, demonstrating that the pro-apoptotic role of dCAF1 in *rbf1* mutant cells is likely general. Importantly, we discovered that decreasing dCAF1 subunits has an impact on the expression of a pro-apoptotic gene *hid,* which was previously identified as a key determinant of cell death in *rbf1* mutant cells. We are currently using chromatin immunoprecipitation to understand the mechanism by which *hid* expression is regulated by dCAF1 to drive cell death susceptibility.

269A

Engulfment receptor, Draper, and its ligand for the clearance of dead cells in the developing *Drosophila* optic lobe. Ryosuke Nakano[1], Masashi Iwamura[1], Akiko Obikawa[1], Ryo Iizuka[1], Hiromi Akagawa[1,2], Hidenobu Tsujimura[1]. 1) Developmental Biology, Tokyo University of Agriculture and Technology, Fuchushi, Tokyo, Japan; 2) Department of Biological Production Science, Tokyo University of Agriculture and Technology, Fuchushi, Tokyo, Japan.

A large number of neuronal cells die through programmed cell death in the developing *Drosophila* optic lobe. This cell death is apoptosis and requires caspase activity. The number of dead cells reaches peak at 24h APF and the dead cells are cleared immediately by 48h APF. However, the mechanism of the clearance has not been defined yet. Here we analyzed the roles of Draper (CED-1/jedi/MEGF-10), which has been implicated to be an engulfment receptor, and its ligand candidates. In *draper* mutant, a greater number of dead cells were observed at 24h APF than wild type and most of them remained to 92h APF. Knockdown of *draper* in glial cells resulted in a greater number of dead cells both at 24h and 48h APF. These facts show that draper is expressed in glia and involved in the clearance. Now, we are examining temporal function of Draper by temporal knockdown of *draper*. Forced expression in glia rescued *draper* mutant phenotype. Next, we examined function of ligand candidates for Draper. CaBP1 and Pretaporter showed only a little role in the clearance. Phosphatidylserine(PS) was suggested to act as a ligand for Draper. Expression of MGF-E8 showed some inhibition of the clearance. Xkr8, a *Drosophila* homologue of PS externalizing factor, played some role in the clearance. We are also analyzing molecules which acts downstream of Draper, shark, crk, mbc, dced-12, and rac1. With other receptor candidates, βIntv and SIMU do not have any roles in the clearance.

270B

Sensitivity to apoptosis is differentially regulated by cell growth pathways. Sarah Neuman, Yunsik Kang, Arash Bashirullah. University of Wisconsin-Madison, Madison, WI.

Cell death and cell growth are two essential processes in both normal development and disease. However, the mechanisms regulating the interplay between apoptosis and cell growth are largely unknown. We demonstrate that apoptotic sensitivity is differentially regulated by activation of growth pathways in *Drosophila*. Activation of the *PI3K/Akt* signaling pathway results in tissue overgrowth and suppression of apoptosis in response to expression of the IAP-antagonist *reaper*. These results are consistent with data from mammalian systems, and led us to investigate whether activation of other cell growth pathways had a similar effect. In contrast to activation of *PI3K/Akt* signaling, knockdown of the tumor suppressor *erupted*, also known as *TSG101*, results in proliferation and increased sensitivity to*reaper*-induced apoptosis. Our results demonstrate that loss of *TSG101* is sufficient to activate apoptosis, even in the absence of an exogenous apoptotic trigger. Altogether, these results demonstrate that sensitivity to apoptosis is differentially modulated by activation of tissue growth pathways, providing novel insights into the interplay between cell growth and cell death. .

271C

Non-autonomous protective effect of dying and undead cells. Tin Tin Su, Angela Delano, Annika Gustafson. MCD Biology, University of Colorado, Boulder, CO.

Last year, we reported a phenomenon wherein induction of apoptosis by a variety of means in wing imaginal discs of *Drosophila* larvae resulted in the activation of an anti-apoptotic microRNA, *bantam*. Cells in the vicinity of apoptotic cells also become harder to kill by ionizing radiation (IR)-induced apoptosis. The protective effect spanned as much as 100 microns away from dying cells. Both *ban*activation and increased protection from IR required receptor tyrosine kinase Tie, which we identified in a genetic screen for modifiers of *ban*. Apoptotic cells showed increased expression of transcriptional reporters for Pvf1 and Pvf2, putative ligands for Tie. We proposed that apoptotic cells activate *ban* in surviving cells through Tie to make the latter cells harder to kill, thereby preserving tissues and ensuring organism survival. These studies, now published, used genuine apoptotic cells, that is, without co-expression of caspase inhibitor p35 (Bilak et al., PLoS Genetics, 2014). We have since investigated whether undead cells, generated by co-expression of caspase inhibitor p35, also confer a similar protective effect. We find that co-expression of p35 in dying cells results in the loss of long-range protection. Instead, we see a stronger, short-range protection that reaches only 3-5 cells away from undead cells. Although undead cells did not activate *ban*, their protective effect is sensitive to *ban* dosage, suggesting that *ban*has a permissive but not an instructive role in this mode of protection. We are currently testing the role of Tie, Pvf1, Pvf2 and other candidate signals in the protective effect of undead cells.

272A

Tousled-like kinase mediates a new type of apoptosis in *Drosophila*. Yu Zhang[1], Lei Liu[1,2]. 1) College of Life Sciences,PKU, Beijing, Beijing, China; 2) Beijing Institute for Brain Disorder and Beijing Tiantan Hospital, Capital Medical University, Beijing 100069, China.

Programmed cell death (PCD) plays an important role in sculpting organisms during development. However, much remains to be

learned about the molecular mechanism of PCD. We found that ectopic expression of *tousled-like kinase (tlk)* in *Drosophila* induced apoptosis. Interestingly, knocking down *tlk* in the eyes reduced the apoptotic death of interommatidial cells (IOCs) and resulted in increase of the number of IOCs. Furthermore, the TLK-mediated apoptosis is likely independent of the canonical caspase pathway and other known caspase-independent pathways.Besides development, knocking down *tlk* was able to suppress calcium overload-induced cell death. Taken together, our results indicate that TLK mediates a new type of caspase-independent apoptosis during eye development and calcium-induced cell death.

273B

Non-autonomous control of nurse cell death by follicle cells in the Drosophila ovary. Albert Mondragon, Allison Timmons, Claire Schenkel, Jon Iker Etchegaray, Jeffrey Taylor, Kim McCall. Boston University, Boston, MA.

Apoptosis, autophagic cell death, and necrosis are the most well characterized forms of programmed cell death; however, alternative cell death pathways are continuously being discovered and characterized. During oogenesis in Drosophila, fifteen nurse cells support the oocyte throughout development. The nurse cells are encompassed by stretch follicle cells, die, and are cleared in a developmentally regulated form of cell death. Inhibiting apoptosis and/or autophagy does not prevent nurse cell death and clearance. Interestingly, we have found that loss of the engulfment machinery (*drpr, Ced-12,* JNK pathway) in the stretch follicle cells disrupts nurse cell death, which has a drastic effect on accumulation of nurse cell debris within the ovary and decreased fecundity. To directly determine the role that stretch follicle cells have in nurse cell death and clearance, we genetically ablated the stretch follicle cells by knocking down the caspase inhibitor, *Diap1*. We found that ablation of stretch follicle cells in late oogenesis led to persisting nurse cell nuclei. Moreover, these egg chambers showed a lack of TUNEL and LysoTracker labeling which are normally observed during nurse cell death. Ablation of stretch follicle cells at earlier stages resulted in dumpless egg chambers, which had a failure in nurse cell actin bundle formation. Taken together our data support a model where the stretch follicle cells non-autonomously control nurse cell dumping, death, and clearance. To elucidate other genes involved in this process we are performing a candidate RNAi screen as well as live imaging to better understand the non-autonomous role of lysosomes and nurse cell death. This example of developmental programmed cell death is a novel model to study non-autonomous cell death and supports the current paradigm shift: apoptosis, autophagy, and necrosis are not the only forms of programmed cell death.

274C

Cell Survival in Muscle Development. Tatevik Sarkissian, Richa Arya, Kristin White. MGH/ Harvard Medical School, Boston, MA.

Cell death is important for normal development and homeostasis. Human health depends on the proper regulation of the cells that are destined to die and those that are required for survival. We are interested in the intrinsic and extrinsic regulators that determine and carry out the death of a particular cell. Specifically, we want to know the role of cell death in myogenesis. We have found that deletion of the major death genes reaper (rpr) and grim results in ectopic muscle fibers. However, the cause of these extra muscle fibers is unclear. Previous work suggests that innervation of muscle fibers is essential for the their growth and formation (Currie and Bate 1995). Furthermore, the number of progenitor founder cells (FC) corresponds to the number of fibers in a muscle (Dutta et. al. 2004). Based on these findings, were are currently investigating whether the cause of extra ventral muscles is due to the survival of ectopic neurons or the formation of ectopic progenitor FCs. Combined, these studies will reveal a mechanistic understanding of how specific muscle fibers are chosen to survive and grow under the control of cell death gene expression.

275A

A heat shock protein regulates caspase-mediated autophagy. Courtney Choutka[1,2], Lindsay DeVorkin[1,2], Nancy Erro Go[1,2], Claire Hou[1], Annie Moradian[3], Gregg Morin[1,3], Sharon Gorski[1,2]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada; 2) The Genome Sciences Centre, BC Cancer Research Centre, Vancouver, British Columbia, Canada; 3) Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

The balance between cell survival and death can be mediated by proteins that have dual-functions in both cell fates and is an area that requires investigation. An unconventional role for a classic apoptotic protein in the regulation of autophagy was discovered. The *Drosophila* effector caspase, Dcp-1, is a positive regulator of starvation-induced autophagy. A proteomics study identified 13 candidate interactors of Dcp-1 that were found to negatively regulate autophagic flux. We chose to further analyze the candidate Hsp83 for its role in autophagy regulation due to its human homolog's links to cell stress and disease (Hsp90). *In vitro* knockdown of Hsp83 lead to increased Lysotracker Green staining and an increased number of autolysosomes suggesting that Hsp83 is a negative regulator of autophagy. This was supported *in vivo* by transheterozygote loss-of-function *Hsp83* mutants that displayed an increase in mid-stage degenerating egg chambers that also stained positive for Lysotracker Red and TUNEL, and had an accumulation of autolysosomes. The pro-form of Dcp-1 associates with partially-localized Hsp83 inside the mitochondria. An *in vitro* knockdown of both Hsp83 and Dcp-1 prevented the accumulation of autolysosomes, similar to Dcp-1, suggesting that Dcp-1 acts downstream of Hsp83 in autophagy regulation. The *Dcp-1;Hsp83* double mutant strain had a phenotype also akin to *Dcp-1*loss-of-function mutants with decreased Lysotracker Red staining. However, mid-stage egg chambers from *Dcp-1;Hsp83* mutants were TUNEL positive, which is similar to the *Hsp83* mutant phenotype and suggests that Hsp83 might activate cell-death through a separate pathway. Additional analyses revealed that *Hsp83* mutants may also have a potential defect in the proteasomal system. These results identify Hsp83 as a new upstream negative regulator of Dcp-1-mediated autophagy and highlight a novel role for a heat shock protein in autophagy modulation. Funding from CIHR MOP-78882 gratefully acknowledged.

276B

Investingating survival mechanisms in transformed *Drosophila* cells following oncogene withdrawal. Ashley Heinaman[1,2], Sathiya Manivannan[1,2], Nanki Hura[1], Molly Josifov[1], Amanda Simcox[1]. 1) Molecular Genetics, The Ohio State University, Columbus, OH; 2) Molecular, Cellular, and Developmental Biology, The Ohio State University, Columbus, OH.

The Ras signaling pathway was first genetically defined in *Drosophila* and many additional pathway genes have been identified in flies. Ras is misregulated in human cancer. A model for Ras-driven mouse pancreatic cancer shows that cells can survive the removal of Ras signaling by entering a dormant state. Genes and pathways involved in the viability of dormant cells could be targets for cancer therapies. Our goal is to use an inducible-Ras tumor model to identify new genes in the Ras signaling pathway and identify those required for dormancy. We have developed a conditional tumor cell line in which proliferation is under the control of oncogenic Ras^{V12} expression induced GeneSwitch-Gal4 (GSR). When Ras^{V12} is expressed (RasON), the GSR cells proliferate but when Ras^{V12} is switched off (RasOFF), the cells stop proliferating, but remain viable and enter a dormant state. RNA-seq analysis identified 300 candidate genes that were specifically expressed in the dormant cells. Interestingly, among these candidate genes, several belonged to the autophagic pathway. We found that two key genes, Atg5 and Lamp-1, required at the beginning and end of the autophagy pathway, respectively, were upregulated in the dormant cells. Immunoblot analysis of these candidates in GSR cells confirms that the dormant cells are autophagic. The role of autophagy in survival of the dormant cells is being tested by blocking autophagy with a known inhibitor of autophagy, chloroquine, as well as RNAi-mediated knockdown of genes required for autophagy. We are also exploring the role other candidate pathways identified through our RNA-seq data to investigate other highly expressed genes in dormant cells.

277C

The NF-kB factor Relish Controls Drosophila Salivary Gland Degradation During Metamorphosis. Anubhab Nandy, Lin Lin, Eric Baehrecke, Neal Silverman. University of Massachusetts Medical School, Worcester, MA.

Drosophila salivary gland degradation occurs during the prepupal to pupal transition, and this phenomenon is a classic system to study developmentally programmed cell death. The peak in ecdysone level that occurs during this transition triggers gland degradation through activation of two different cell death pathways, apoptosis and autophagy. Here, we show that the NF-kB factor Relish, which is best known for its pivotal role in the *Drosophila* immune response, also influences cell death during salivary gland degradation. In particular, salivary gland fragments persist in *Relish* mutant animals. In the context of the immune response, Relish is the major transcription factor activated by the IMD pathway following bacterial infection. Interestingly, several IMD pathway components are induced in the salivary gland during metamorphosis, including PGRP-LC, Relish, and several anti-microbial peptides. All available mutants affecting IMD signaling were similarly analyzed for salivary gland degradation phenotypes. Only *Relish* and *PGRP-LC, PGRP-LE* double mutants displayed persistent gland fragments; none of the other IMD pathway components appear to play a role in salivary gland degradation. Genetic and cell biological analyses demonstrate that Relish influences salivary gland degradation by controlling the autophagy pathway, but not the apoptotic pathway. Ectopic expression of an active form of Relish results in the activation of autophagy and premature destruction of salivary glands. On the other hand, overexpression of six individual AMP genes failed to induce gland degradation, suggesting that Relish-dependent autophagy and salivary gland degradation is independent of antimicrobial peptides. Overall, this study establishes the importance of the NF-kB factor Relish in developmentally controlled cell death, through the regulation of autophagy. Further investigations aim to identify the underlying mechanisms by which Relish controls autophagy.

278A

Mitochondrial fission plays a key role in neuronal necrosis downstream of the epigenetic alteration in *Drosophila*. Lianggong Ding[1], Lei Liu[1,2]. 1) State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Peking University, Beijing 100871, China; 2) Beijing Institute for Brain Disorder and Beijing Tiantan Hospital, Capital Medical University, Beijing 100069, China.

Neuronal necrosis induced by calcium overload has been considered as a stochastic process lacking genetic regulation. However, recently we discovered that neuronal necrosis was mediated by a cascade of epigenetic events in *Drosophila* and mammalian neurons. This epigenetic change initiated from phosphorylation of H3 serine 28 (H3S28ph) by JIL-1/MSK1/2, then, displacement of polycomb repressive complex 1 (PRC1), which led to disinhibition of Trithorax (Trx). The downstream target of these epigenetic modifications is still unclear. Here, we devised our experiments to search for the downstream targets. We found that blocking mitochondria fission by loss-of-function of Drp1, a regulator of mitochondrial fission, showed rescue effect against necrosis. In addition, excessive mitochondria fission indeed occurred in the neurons undergoing necrosis. To determine whether the mitochondria fission was downstream of the epigenetic modifications, we examined the mitochondrial morphology under the mutant background of Trx in the necrotic flies. The result showed that mitochondrial fission was blocked by the Trx mutant, suggesting the mitochondrial damage occurred downstream of the epigenetic pathway.Our results implicate that mitochondrial fission is important regulator of necrosis downstream of the epigenetic cascade. The mechanism about how epigenetic pathway regulates mitochondrial fission is under our investigation.

279B

Genetic screen to identify new mechanisms of spreading apoptosis induced by necrotic neurons in *Drosophila*. Lin Hou[1], Rong Cai[1], Lei Liu[1,2]. 1) State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Peking University, Beijing 100871, China; 2) Beijing Institute for Brain Disorder and Beijing Tiantan Hospital, Capital Medical University, Beijing 100069, China.

Neurons undergoing necrosis often promote death to their neighboring cells, or spreading death. Spreading death commonly takes place in various neurodegenerative diseases. However, the interactions between necrotic cells and their neighbors are less understood. Previously, we have established a *Drosophila* model to study these interactions. We expressed a Ca^{2+}-permeable leaky channel (*UAS-GluR1^{Lc}*) to induce necrosis selectively in three of eight photoreceptor neurons in each ommatidium of *Drosophila* eye driven by *sev-Gal4* (the progeny flies are simplified as *sev>GluR1^{Lc}*). Using this model, we have determined that the neuronal necrosis could indeed promote spreading death in neurons. Further, we found that caspase-dependent apoptosis and JNK-dependent apoptosis accounted for ~8% and ~47% of the spreading apoptosis, respectively. Interestingly, ~45% of spreading apoptosis was still undetermined. Here, we devised our experiments to screen for potential new mechanisms of spreading apoptosis. By P-element screens, we found that a loss-of-function mutant of *TOR* and a hypermorph of *atg6* rescued the eye defect of *sev>GluR1^{Lc}* flies. Moreover, decreased AO staining was observed in the eye disc, indicating reduction of surrounding apoptosis in these mutants. Our preliminary data suggests that they are likely play a key role to regulate the unknown type of spreading apoptosis. Because *TOR* and *Atg6* are known to regulate autophagy, we are investigating the role of autophagy and related mechanisms in spreading apoptosis.

280C

The Roles of Cyclins A, B, and B3 in *Drosophila* Female Meiosis. Mohammed Bourouh, Rajdeep Dhaliwal, Ketki Rana, Sucheta Sinha, Zhihao Guo, Andrew Swan. Biological Sciences, University of Windsor, Windsor, Ontario, Canada.

Meiosis, compared to mitosis, is a more complex and less understood nuclear division, yielding 4 genetically distinct haploid cells. As in mitosis, meiosis is regulated by Cyclin Dependent Kinases (Cdks). Cdks are activated when bound to their cyclin partners. The type of cyclin bound confers the substrate specificity of the Cdk, but some redundancies exist within cyclin families. In *Drosophila*, there are three mitotic cyclins, Cyclin A, B, and B3. Previous work in mitosis has shown that *cyclin A* mutants are lethal, reflecting a possible role in G2. Cyclin B is redundant with Cyclin A in entry to mitosis and nuclear envelope breakdown. *Cyclin B* and *cyclin B3* single mutants are viable, whereas *cyclin B* and *cyclin B3* double mutants are lethal, reflecting the redundancy between these two cyclins in mitotic progression. While the individual and redundant roles of the Cyclin-Cdk complexes are known in mitosis, their roles in meiosis are not clear. Cyclin B3 has been implicated in female meiosis; however, a role for Cyclin B and Cyclin A in meiosis has not yet been determined. This work focuses on characterizing the roles and redundancies between the mitotic cyclins in female meiosis. We used RNAi to study roles of Cyclin A and Cyclin B in meiosis, thus circumventing the zygotic requirement of Cyclin A, and pre-meiotic requirement of Cyclin B. For Cyclin B3, we used previously described mutant alleles. In addition to examining single mutants, pairwise and triple knockdowns were performed to examine the redundancy between the cyclins in meiosis. We found that all three cyclins have distinct functions in meiosis. We show that all three cyclins are involved in nuclear envelope breakdown, with Cyclin A having the most important role, Cyclin B in polar body formation, and Cyclin B3 in anaphase progression of meiosis I and II. We also show that, Cyclin A and Cyclin B3 show greater redundancy than Cyclin B and Cyclin B3, which is contradictory to what is seen in mitosis. Our results indicate that the cyclins appear to have more specialized roles in meiosis than in mitosis, as all three cyclins are necessary for proper meiosis progression in females.

281A

The mitotic role of SCF-Skp2 in maintaining genome stability. Nilanjana Das, Biju Vasavan, Andrew Swan. Dept. of Biological Sciences, University of Windsor, Windsor, Ontario, Canada.

Skp2 is a substrate recognition component of the E3 ubiquitin ligase SCF complex and is an established oncogene. It is found to be frequently overexpressed in almost all cancer types. Skp2 has also been shown to cooperate with activated Ras to transform normal cells to malignant cells. Skp2 targets p27, a cyclin dependent kinase inhibitor and a known tumor suppressor for ubiquitilation and later degradation. Skp2 overexpression in most cancer types directly correlates with lower p27 level. This results in cells moving precociously into S phase from G1. Due to the oncogenic nature of Skp2 in a wide range of cancer cell types, Skp2 has been proposed to be an attractive clinical target in the fight against cancer. While research so far has concentrated on this oncogenic role of Skp2 during the G1-S transition, research in our lab reveals that Skp2 also has an important G2–M role which appears to be tumor suppressive rather than oncogenic. We previously generated Skp2 null flies in our lab and showed that loss of Skp2 causes polyploidy and genome instability. In this study we found that loss of Skp2 results in the activation of the Spindle Assembly Checkpoint (SAC) leading to mitotic delay or prometaphase arrest. This appears to represent a polyploidy sensing checkpoint. We also observed a partial failure of Skp2 null cells to enter mitosis, apparently due to premature degradation of mitotic cyclins in Skp2 mutants. Skp2 interacts genetically and physically with Cyclin A and Cyclin B3 but not with Cyclin B. We are currently investigating a possible role of Skp2 in protecting mitotic Cyclins from another E3 ubiquitin ligase, the Anaphase Promoting Complex (APC/C) in the G2 phase of the cell cycle.

282B

Characterization of a *Drosophila* ortholog of the Cdc7 kinase: Differential requirement for Dbf4/Chiffon in distinct DNA replication programs within *Drosophila*. Robert Stephenson[1], Marcus Hosler[1], Navnath Gavande[2], Arun Ghosh[2,3], Vikki Weake[1,3]. 1) Department of Biochemistry, Purdue University, West Lafayette, IN; 2) Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, IN; 3) Center for Cancer Research, Purdue University, West Lafayette, IN.

Cdc7 is a serine-threonine kinase that phosphorylates components of the pre-Replication Complex during DNA replication initiation. Cdc7 is highly conserved, and Cdc7 orthologs have been characterized in organisms ranging from yeast to humans. Cdc7 is activated specifically during late G1/S-phase by binding to its regulatory subunit, Dbf4. *Drosophila melanogaster* contains a Dbf4 ortholog, Chiffon, which is essential for chorion loci amplification in *Drosophila* egg chamber follicle cells. However, no *Drosophila* ortholog of

Cdc7 had been characterized prior to this study. *Drosophila* contains two Cdc7 orthologs encoded by the ubiquitously expressed gene *l(1)G0148*, and the testes-specific gene *CG5790.* Here we show that co-expression of *l(1)G0148* (Cdc7) and *Chiffon* complements the mitotic growth defect in yeast containing a temperature-sensitive *CDC7* mutant, while co-expression of *CG5790* and *Chiffon* does not. We further show that *Drosophila* Cdc7 and Chiffon physically interact, and can be co-purified from insect cells. In addition, *Drosophila* Cdc7 phosphorylates the known Cdc7 substrates Mcm2 and histone H3 *in vitro,* and Cdc7 kinase activity is stimulated by Chiffon and inhibited by the Cdc7-specific inhibitor XL413. *Drosophila* egg chamber follicle cells deficient for Cdc7 have a defect in two types of DNA replication, endoreplication and chorion gene amplification. Interestingly, follicle cells deficient for Chiffon have a defect in chorion gene amplification, but still undergo endoreplication. Our results show that *Drosophila* Cdc7 interacts with Chiffon to form a functional Dbf4 Dependent Kinase (DDK) complex, and that Cdc7 is necessary for DNA replication in *Drosophila* egg chamber follicle cells. Additionally, we show that Chiffon is differentially required for distinct forms of DNA replication within *Drosophila,* raising the possibility that *Drosophila* may contain additional activating subunits for Cdc7 that have yet to be identified. .

283C

***Drosophila RNase Z^{L}* links mitochondrial RNA processing and cell cycle progression. Xie Xie**, Veronica Dubrovskaya, Edward Dubrovsky. Biological Sciences, Fordham University, Bronx, NY.

Drosophila RNase Z^{L} (*dRNaseZ*) gene encodes a highly conserved protein responsible for both nuclear and mitochondrial tRNA maturation. Although knockout of the *RNase Z^{L}* gene has shown to damage the processing of both populations of tRNAs, no study has functionally proven the mitochondrial localization of RNase Z^{L}, nor has examined the cellular function of mitochondrial RNase Z^{L}. In this study, we genetically knocked out the intra-mitochondrial function of *Drosophila RNase Z^{L}* (*RNZ$^{\Delta MTS}$*). We found that the mitochondrial activity of dRNaseZ is required for the maturation of all three types of mitochondrial transcripts, tRNA, mRNA, and rRNA. Losing mitochondrial dRNaseZ (mt-dRNaseZ) significantly damages the activity of electron transport chain, leads to reduced mitochondrial ATP production and increased ROS. Using mitotic recombination and conditional rescue techniques, we showed that mitotic *RNZ$^{\Delta MTS}$* cells are proliferation-impaired, have increased DNA damage and display cell cycle delay at G2/M transition. We further showed that rescue with antioxidant N-acetyl cysteine (NAC) decreases ROS level and DNA damage, and alleviates the proliferation defect, indicating that ROS and oxidative DNA damage is the primary cause of proliferation defects in *RNZ$^{\Delta MTS}$* cells. Our results demonstrate the essential role of mt-dRNaseZ in mitochondrial transcript processing and functionality. In addition our data indicate a novel pathway that modulates G2/M transition through endogenous ROS production.

284A

***Drosophila* Mcm10 is required for female reproductive success. Michael C. Reubens**, Sidney Bedsole, Megan Biller, Lucas Hopkins, Elizabeth T. Ables, Tim W. Christensen. Department of Biology, East Carolina University, Greenville, NC.

Efficient replication of the genome and the establishment of endogenous chromatin states are processes essential to eukaryotic life. It is well documented that Mcm10 is intimately linked to both of these important biological processes; therefore, it is not surprising that *Mcm10* is commonly misregulated in many human cancers. Most of the research regarding the biological roles of Mcm10 has been performed in single-cell or cell-free *in-vitro* systems. Though these systems are informative, they are unable to provide information on the cell-specific function of Mcm10 in the context of the tissue and organ systems that comprise multicellular eukaryotes. We therefore sought to identify the potential biological functions of Mcm10 in the context of a complex multicellular organism by continuing our analysis in *Drosophila*using our previously described *Mcm10^{d08029}* truncation allele in conjunction with three novel hypomorphic alleles. Observation of embryonic nuclear morphology and quantification of embryo hatch rates reveal that maternal loading of *Mcm10* is required for embryonic nuclear stability, and suggest a role for Mcm10 post zygotic transition. Contrary to the essential nature of Mcm10 depicted in the literature, it does not appear to be required for adult viability in *Drosophila* as long as the embryonic requirements are met. Although not required for adult somatic viability, analysis of fecundity and ovarian morphology in mutant females suggest that Mcm10 plays a role in maintenance of the female germline; however, male mutants do not display significant impacts on overall fecundity. The mechanistic role of Mcm10 in the cellular biology of female specific germline maintenance and embryogenesis remains unclear, but will be addressed in future studies in our lab. These results mark the first data linking Mcm10 to female specific reproductive biology in multicellular eukaryotes, and demonstrates the importance of working within the context of a multicellular system in order to explore the potential functions proteins carryout in the cellular hierarchy characteristic of multicellular life. .

285B

Determining the role of FancM, Blm, and HelQ helicases in the repair of double strand breaks. Julie Cox, Adam Thomas, Mitch McVey. Tufts University, Medford, MA.

In Drosophila, DNA double-strand breaks that occur in the soma and the pre-meiotic germline are frequently repaired by homologous recombination via synthesis-dependent strand annealing (SDSA). Several different helicases, including Blm, FancM, and HelQ, are involved in SDSA. Their importance is highlighted by the genome instability and cancer that occurs in their absence. However, their exact roles and how they interact with each other are not well understood. Here, we report the results of experiments designed to elucidate the in vivo roles of these helicases.

We constructed single and double mutants lacking Blm and/or HelQ and tested their ability to carry out SDSA repair using a well-characterized site-specfic gap repair assay. Similar to published reports, we observed that blm mutants were defective in SDSA repair,

with short repair synthesis tract lengths and a high frequency of deletions flanking the break site. Flies lacking HelQ were also unable to carry out SDSA repair proficiently, but did not display a deletion-prone phenotype. Interestingly, loss of HelQ suppressed the deletion-prone repair of the Blm mutants, suggesting that these two helicases play different roles during SDSA and that a third helicase might be used in the absence of Blm.
Because both FancM and Blm can unwind D-loop recombination intermediates in vitro, we tested whether FancM is involved in SDSA. Indeed, fancM mutants had a decreased ability to complete SDSA repair, but flanking deletions were not observed. Approximately 20% of fancM mutant repair events resulted from immediate end-joining, suggesting that FancM may be important for the initiation of SDSA. Finally, repair events from fancM helQ flies exhibited extremely short DNA synthesis tracts, consistent with independent functions for these two helicases during SDSA. Our results suggest that the Blm and FancM helicases play partially overlapping roles during SDSA repair, but that Blm, HelQ, and FancM also have independent functions in D-loop processing and regulation of HR repair. .

286C

The NuA4 complex coordinates proper cell cycle gene expression with phase, which impacts the transition to a post-mitotic state. Kerry Flegel, Olga Grushko, Kelsey Bolin, Ellen Griggs, Nicholas Rachmaninoff, Laura Buttitta. University of Michigan, Ann Arbor, MI.

Developmental signals integrate with the cell cycle machinery to coordinate proliferation and differentiation *in vivo*. This is apparent during the process of terminal differentiation, which is often coordinated with a prolonged or permanent cell cycle exit. Robust and synchronous silencing of cell cycle gene expression is critical to the proper timing of cell cycle exit, but the molecular details of this remain unresolved. We have found that the well-conserved multi-subunit complex, nucleosome acetyltransferase of histone H4 (NuA4), plays an important role in the proper timing of cell cycle exit associated with differentiation in *Drosophila*. Our *in vivo* inhibition of NuA4 function delays cell cycle exit and misregulates cell cycle gene expression in terminally differentiating cells, without preventing or delaying terminal differentiation. Our data indicates that the NuA4 complex acts during the final cell cycle to initiate proper silencing of cell cycle genes *in vivo*. We find that NuA4 plays a role in coordinating cell cycle gene expression with the appropriate cell cycle phases. We speculate that NuA4 inhibition disrupts proper phasing to prolong S/G2/M duration, thereby delaying the transition to a permanent non-cycling state. Our data suggest that NuA4 acts upon the transcriptional oscillation of cell cycle genes to affect proper cell cycle phase duration, overall cell cycle length, and the timing of cell cycle exit.

287A

Using gene expression profiling and quantitative proteomics to study E2F/Dp function in *Drosophila*. Ana Guarner, Robert Morris, Michael Korenjak, Myriam Boukhali, Sridhar Ramaswamy, Wilhelm Haas, Nick Dyson. MGH Cancer Center (Massachusetts General Hospital / Harvard Medical School). 149 13th Street, 02129. Charlestown, MA. USA.

E2F/RB proteins regulate cell proliferation and differentiation and have been conserved from *Drosophila* to humans. The canonical E2F transcription factor is a heterodimer of E2F and DP subunits, in which both subunits are required for sequence-specific DNA binding activity. *Drosophila* contains two E2F proteins but just a single form of DP. dE2F/dDP can function as repressors when associated with RB proteins. In mammalian cells E2F activity results from the integrated activity of multiple E2F/RB genes, however this network is less complicated in *Drosophila*. Notably, the mutation of *dDp* provides a unique opportunity to examine the consequences of the complete elimination of this network. $dDp^{-/-}$ larvae die in pupal stages. In these mutants, the stable association of RBF1 with chromatin is completely abolished (Korenjak et al., 2012) and mitochondrial function is compromised (Ambrus et al., 2013).
To understand the consequences of the complete loss of E2F activity we have performed a semi-quantitative proteomic analysis of WT and *dDp* larvae. TMT-Quantitative Mass Spectrometry enabled us to compare the relative levels of >5000 proteins in WT and *dDp* larvae. We profiled whole cell extracts, as well as extracts that had been fractionated into cytoplasmic, soluble nuclear and insoluble nuclear proteins. Our results reveal significant differences between the WT and *dDp* proteome, including extensive alterations in proteins involved in mitochondrial function, lipid metabolism and chromatin compaction. For identifying the molecular drivers of the *dDp* phenotype we have compared the proteome changes with changes in RNA levels. Interestingly, the transcriptional profile of *dDp* mutants gives an incomplete picture of the *dDp* proteome. This, in part, may be due to changes in processes that are indirectly affected by dDP. We have integrated this data with ChIP-chip data to determine which of the direct targets of E2F/DP/RBF proteins display altered transcription and altered protein levels in *dDp* mutant larvae. .

288B

The role of E2F in regulating muscle growth is necessary and sufficient for fly viability. Maria Paula Zappia, Maxim V. Frolov. Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL.

The E2F transcription factors (E2Fs) have been well established as key regulators of cell cycle progression, and cell death.
In *Drosophila*, there are only two *E2f* genes, encoding for an activator, E2F1, and a repressor, E2F2. *E2f* double mutants develop relatively normally but eventually die at pupal stage. The cause of death remains largely unknown, even though E2F functions have been extensively studied. Here we demonstrate that *E2f* double mutant died because of the loss of E2F in muscles, which affected skeletal muscle growth. Strikingly, the lethality phenotype in *E2f* double mutants was rescued by restoring E2F activity specifically in the adult skeletal muscle. Conversely, depletion of E2Fs specifically in muscle led to lethality at pupal stage, while E2F inactivation in other tissues did not. Therefore, the function of E2Fs in adult skeletal muscle is necessary and sufficient for viability in *Drosophila*.
The loss of E2Fs resulted in a considerable reduction in the size of the indirect flight muscles. E2Fs are well known for their central role in cell cycle progression. However, unexpectedly, myoblast proliferation as well as myoblast fusion were not altered, thus supporting the

notion that depletion of E2F affects primarily muscle hypertrophic growth. We showed that E2Fs are required to coordinate the temporal regulation of muscle-specific gene expression. The expression of several myogenic genes was only affected during late muscle differentiation in E2F-depleted muscles. E2F proteins and other components of the complex, including RBF1, were significantly enriched upstream of the *Mef2* gene, a key myogenic transcription factor that activates differentiation, and the *Flightin* gene, a structural component of flight muscles.
Overall, our findings support a model in which E2Fs tightly control muscle growth and integrity through the direct transcriptional regulation of myogenic genes during late muscle differentiation. The novel role of E2Fs in skeletal muscle differentiation is necessary and sufficient for development, thus uncovering a novel postmitotic role for E2Fs.

289C

Scalloped and Yorkie are required for cell cycle re-entry of quiescent cells after tissuge damage. J.H. Meserve[1], R.J. Duronio[1,2,3,4]. 1) Curriculum in Genetics & Molecular Biology, UNC, Chapel Hill, NC; 2) Integrative Program for Biological and Genome Sciences, UNC, Chapel Hill, NC; 3) Departments of Biology and Genetics, UNC, Chapel Hill, NC; 4) Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

Regeneration of damaged tissues typically requires a population of proliferatively active stem cells. How damaged tissue is regenerated in post-mitotic tissues lacking a stem cell population is less well understood. We used a genetic screen in the developing *Drosophila melanogaster* eye to investigate the mechanisms that trigger quiescent cells to re-enter the cell cycle and proliferate in response to tissue damage. We discovered that Hippo signaling regulates compensatory proliferation after extensive cell death in the eye imaginal disc. Scalloped and Yorkie, transcriptional effectors of the Hippo pathway, drive expression of Cyclin E to induce cell cycle re-entry in cells that normally remain quiescent in the absence of damage. Ajuba, an upstream regulator of Hippo signaling that functions as a sensor of epithelial integrity, is also required for cell cycle re-entry. Thus, in addition to its well-established role in modulating proliferation during periods of tissue growth, Hippo signaling maintains homeostasis by regulating quiescent cell populations affected by tissue damage.

290A

CG10126, a calcium-binding microtubule-associated protein, promotes mitosis during Drosophila development. Q. Nie, S. Spencer. Saint Louis University, Saint Louis, MO.

The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase that regulates signaling pathways critical for development and proliferation in *Drosophila*. EGFR activity is also highly upregulated in a number of human cancers, making identification of its downstream targets important for understanding both basic cellular processes and disease states. We have identified CG10126, a*Drosophila* ortholog of the human Calcyphosine-like protein, as a transcriptional target of EGFR signaling. Calcyphosine encodes a calcium-binding protein upregulated in several human cancers, but little is known of its function. Here we examine the role of CG10126 during *Drosophila* development. To examine CG10126's role in developing cells, we expressed RNA interference constructs at various points in development. Reducing the level of CG10126 in eye imaginal discs produced adults with small eyes, suggesting that cell proliferation may be decreased; consistent with this, the number of P-Histone3-positive cells was diminished in these tissues. Similarly, reducing CG10126 ubiquitously in embryos was generally lethal; rare escapers were dramatically smaller in size. To further examine the potential role of CG10126 in promoting cell division, we used immuneprecipitations from S2 cell lysates and mass spectrometry to identify binding partners of CG10126 in the presence or absence of calcium. Specifically in the presence of calcium, CG10126 bound α- and β-tubulin and several spindle assembly proteins, suggesting that it may influence formation of the mitotic spindle. In keeping with this, embryos with reduced CG10126 show a diminished number of mitotic nuclei and abnormal spindle structures. Calcium is indispensable for cell cycle regulation (Roderick, H. L. & Cook, S. J. Nat Rev Cancer 8, 361-375, (2008)); our results suggest that calcium waves present during mitosis may enable CG10126 to influence microtubule dynamics and spindle assembly. Together, our studies suggest that CG10126, a downstream target of EGFR signaling, is a microtubule-associated protein that affects cell proliferation by regulating mitotic spindle formation.

291B

Protein phosphatase 1 antagonizes Aurora B kinase in maintaining chromosome structure and cohesion in Drosophila oocytes. Arunika Das, Rachel A. Battaglia, Kim S. McKim. Waksman Institute, Rutgers University, Piscataway, NJ.

Accurate segregation in the germline is achieved by formation of a specialized microtubule based structure called the spindle. In female meiosis of most animals including Drosophila, a bipolar spindle assembles without the guidance of centrosomes. In this system, knockdown of the master cell cycle regulator Aurora B kinase results in a failure to assemble spindle microtubules and kinetochores. We have further found that if an Aurora B inhibitor is added after spindle assembly is complete, the microtubules disassemble and kinetochore proteins do not localize. This indicates that the spindle assembly factors require continual phosphorylation for accurate segregation to occur due to either the presence of multiple phosphatases or protein exchange. Hence, protein phosphatases may play important roles in spindle assembly and chromosome segregation. We have examined the role of the serine/threonine protein phosphatase 1 (PP1) in female meiotic chromosome segregation and spindle assembly. In PP1 depleted oocytes, we observe a gross disorganization of spindle microtubules. In addition the karyosome, a structure into which all the chromosomes are compacted, is dispersed into several masses with loss of sister-centromere cohesion. Localization of kinetochore proteins like Spc105R (KNL1 homolog), however, is unaffected by loss of PP1. We have also found that some but not all of these phenotypes are dependent on active Aurora B. The karyosome defect and loss of cohesion in oocytes lacking PP1, is rescued by addition of an Aurora B inhibitor.

Furthermore the loss of kinetochore protein Spc105R at centromeres, upon adding the inhibitor of Aurora B, is rescued by the loss of PP1. These results suggest that PP1 antagonizes Aurora B for maintaining cohesion, karyosome integrity and kinetochore protein localization. However, the complete loss of the meiotic spindle caused by the Aurora B inhibitor is not restored by loss of PP1. Thus, other phosphatases may negatively regulate spindle assembly and/or Aurora B-dependent phosphorylation is required to maintain incorporation of spindle associated proteins throughout meiosis.

292C

Synaptonemal complex assembly is regulated by multiple cohesion complexes in Drosophila meiosis. Mercedes Gyuricza, Kathryn Landy, Vandana Apte, Kim McKim. Waskman Institute, Rutgers University, Piscataway, NJ.

Cohesin, a ring-like structure comprised of four subunits, has been extensively studied in mitosis and is involved in holding sister chromatids together. During meiosis, sisters are held together by cohesion and the homologous chromosomes by the synaptonemal complex (SC). All cohesin complexes have two SMC subunits, a kleisin and a stromalin. Organisms such as mice have many meiosis specific cohesin complexes with unknown functions. From previous results it is evident that Drosophila has multiple cohesin complexes in meiosis as well. This is hypothesized because the four canonical subunits of the complex (SMC1, SMC3, Rad21, and Stromalin) have different effects on SC assembly when knocked out. These multiple phenotypes indicate that the canonical complex may not be the complex associated with SC assembly. SC is also dependent upon C(2)M, a protein known to have homology to the kleisin family and found at the chromosome axis. Because *c(2)M* mutants have a patchy SC phenotype that is less severe than in *SMC* mutants (no SC assembly), we propose that C(2)M functions as the kleisin in some but not all of the cohesin complexes in Drosophila meiosis. *stromalin* (SA) RNAi also results in an intermediate, patchy, SC phenotype indicating that they may be functioning in a complex together. We are currently validating the intermediate phenotype by making a null mutation in*SA*. ORD is another protein involved in SC assembly and cohesion, found at the chromosome axis. *ord* mutants, however, lack initial foci of SC but form full threads. This indicates that it may be involved with a different cohesin complex. Double mutant analysis of *c(2)M*; *ord* reveals no SC assembly similar to the phenotype of the SMCs, consistent with two different cohesin complexes mediating SC assembly. We are currently characterizing the components of each of the cohesin complexes. We also have evidence that indicates that complexes containing C(2)M are dynamic and are only required for homolog interactions whereas the complex with ORD is required for sister interactions. We are currently testing this model with mutants of other cohesin components and determining if they are static or dynamic. .

293A

Increased Oxidative Stress in Drosophila Oocytes Leads to Loss of Meiotic Cohesion and Chromosome Segregation Errors. A.T. Perkins, E.M. Morse, T.M. Das, C.A. Jeffreys, B.A. Toffey, L.A. Oberg, S.E. Bickel. Dartmouth College, Hanover, NH.

During meiosis, cohesion along the arms of sister chromatids keeps recombinant homologs physically attached until anaphase I and premature loss of arm cohesion can lead to missegregation of homologs during meiosis I. In humans, meiotic segregation errors that produce aneuploid gametes are the leading cause of miscarriages and birth defects and these errors increase dramatically during a woman's thirties. A growing body of evidence suggests that meiotic cohesion deteriorates as oocytes age and contributes to the maternal age effect. One hallmark of aging cells is an increase in oxidative damage caused by reactive oxygen species (ROS) produced primarily in the mitochondria. Therefore, increased levels of oxidative stress in older oocytes may be one of the factors that lead to premature loss of cohesion and segregation errors. We tested this hypothesis by increasing the level of oxidative stress in Drosophila oocytes and measuring the effect on nondisjunction (NDJ). We utilized an inducible GAL4-UAS RNAi strategy to reduce the ROS scavenger Superoxide Dismutase (SOD) in the female germ line during meiotic prophase, after normal cohesion establishment. We observed a small but significant increase in NDJ when either SOD1 (cytoplasmic) or SOD2 (mitochondrial) was knocked down. In Drosophila oocytes, the achiasmate segregation system not only helps to ensure the accurate segregation of non-recombinant homologs, but also homologs that have lost their chiasmata due to loss of arm cohesion. Therefore, to accurately assess the effect of oxidative stress on the segregation of meiotic chromosomes, we knocked down SOD in a $mtrm^{+/-}$ heterozygous background in which the achiasmate system was disabled. In these tests, the effect of SOD knockdown on meiotic NDJ was considerably more robust. Furthermore, we observed a significant increase in the number of missegregating recombinant chromosomes, indicating that chiasmata destabilitzation due to loss of cohesion resulted when oxidative stress was increased. Together these results support the model that accelerated loss of cohesion in aging oocytes is caused, at least in part, by increased levels of oxidative stress.

294B

Organization of the dynamic centrosome structure in rapidly dividing embryos. Dorothy A. Lerit[1], John S. Poulton[2], Holly A. Jordan[1], Carey J. Fagerstrom[1], Brian J. Galletta[1], Karen M. Plevock[1,2], Mark Peifer[2], Nasser M. Rusan[1]. 1) Cell Biology and Physiology Center. NHLBI, NIH. Bethesda, MD; 2) Department of Biology. University of North Carolina. Chapel Hill, NC.

Centrosomes are the microtubule-organizing centers (MTOCs) of most eukaryotic cells, and they serve vial roles throughout the cell cycle. A protein-rich matrix, the pericentriolar material (PCM), is the essential determinant for the MTOC activity of the centrosome. How centrosomes orchestrate the dynamic organization of the PCM structure throughout the cell cycle is a little understood process. Elucidating how PCM dynamics are regulated is critical to understanding how centrosome dysfunction contributes to pathology, such as microcephaly and cancer.

Using clonal mutant analysis in *Drosophila* embryos, we have identified a novel mechanism required for interphase PCM organization. Our data indicate Pericentrin-like-protein (PLP) is required for normal centrosome structure and activity. Loss of PLP results in dramatic PCM dispersal, which impairs centrosome separation, microtubule organization, and cell cycle progression. Ultimately, these defects

result in DNA damage, nuclear fallout, and embryonic lethality. Live and super-resolution imaging reveals that PLP localizes to novel, radial extended structures that are present only during interphase and are reminiscent of mammalian centriolar satellites. We show PLP satellites define the interphase PCM structure and are required to scaffold the key PCM components Centrosomin (Cnn) and γTubulin. Cnn is also known to function as a PCM scaffold, and mutations in the human orthologs of PLP and Cnn both cause microcephaly, raising the possibility that PLP and Cnn may function cooperatively to regulate centrosome structure. In support of this model, we have mapped a direct interaction between PLP and Cnn. Disruption of this interaction specifically abolishes PLP localization to satellites and produces a phenocopy of the PCM disorder observed in *plp* germline clones. Collectively, our data provide mechanistic insight into the proper organization of interphase PCM by a molecular scaffold comprising PLP satellites and Cnn that is essential for normal cell division and early development.

295C

The microcephaly protein Wdr62/CG7337 is required to maintain centrosome asymmetry in *Drosophila* neuroblasts. Anjana Ramdas Nair[1], Priyanka Singh[1], David Rodriguez Crespo[2], David Salvador[1], Borris Egger[2], Clemens Cabernard[1]. 1) Biozentrum,University of Basel,Klingelbergstrasse 50-70,4056 Basel,Switzerland; 2) Department of Biology,University of Fribourg,Chemin du Musée 10,CH-1700, Fribourg,Switzerland.

Centrosome asymmetry has been implicated in stem cell fate maintenance in flies and vertebrates. *Drosophila* neuroblasts, the neural precursors of the central nervous system, contain molecularly and physically asymmetric centrosomes. For instance, the apical daughter centrosome maintains stable microtubule organizing center (MTOC) activity and remains tethered to the apical cortex throughout the cell cycle. The basal mother centrosome, however, loses MTOC activity and only regains it during prophase. This centrosome asymmetry is important for centrosome positioning, spindle orientation and centrosome segregation during asymmetric cell division.
In a gene candidate approach, we identified the uncharacterized gene *CG7337,* the fly ortholog of WDR62, as a regulator of centrosome asymmetry during interphase. We generated CRISPR and Flp-FRT mediated loss of function alleles of *CG7337* (henceforth *wdr62*) and used live imaging to investigate centrosome asymmetry in this mutant background. In *wdr62* mutant neuroblasts both centrosomes lose MTOC activity during interphase, resulting in two untethered centrioles. *wdr62* mutants fail to downregulate pericentrin-like protein (PLP) on the apical centrosome, leading to the downregulation of Polo and hence loss of MTOC activity. We further found that *wdr62* mutants display cell cycle delay and concomitantly, a decrease in brain size. Using MiMIC, we tagged Wdr62 at its endogenous locus and found that Wdr62 is enriched on the apical centrosome at interphase.
These results suggest that Wdr62 is a centrosomal protein, required to maintain apical MTOC activity during interphase by regulating Polo localization through PLP. Wdr62 is also necessary for timely mitotic entry of neuroblasts, ensuring normal development of the brain.

296A

3D-structured illumination microscopy of Centriolar and Centrosomal proteins in *Drosophila melanogaster* neuroblasts. Anjana Ramdas Nair, Priyanka Singh, Alexia Loynton-Ferrand, Clemens Cabernard. Biozentrum, University of Basel, Klingelbergstrasse 50-70, 4056 Basel, , Switzerland.

Centrosomes are the microtubule organizing centers (MTOCs) of eukaryotic cells, consisting of a pair of centrioles, surrounded by a matrix of pericentriolar proteins. *Drosophila* neuroblasts consist of physically and molecularly asymmetric centrosomes. This asymmetry is necessary for correct centrosome positioning, spindle orientation and centrosome segregation. However, the mechanisms underlying the establishment of centrosome asymmetry are incompletely understood.
Here, we have used super-resolution 3D-structured illumination microscopy (3D-SIM) to better understand (1) the spatial relationship of key pericentriolar and centriolar components of *Drosophila*neuroblast centrosomes at different cell cycle stages, (2) the timing of nascent centriole formation and (3) the establishment of centrosomal asymmetry.
Centriolar proteins such as Sas-6 and Ana-2 were detectable as a dot at the core of the centriole, consistent with their role as cartwheel components. We observed that other centriolar proteins form a ring- like structure of varying densities and diameter around the cartwheel along the length of the centriole with the following order: Sas4, Polo, bld10, Asterless (Asl) and Pericentrin-like protein (PLP).We also found that Sas-6 formed a dot next to the older centriole soon after centriole separation at interphase, indicating that Sas-6 is an early marker for the site of nascent centriole cartwheel formation. Sas-4 starts localizing on the nascent centriole wall during late interphase followed by Bld10 and Asl at Prometaphase. Remarkably, Polo starts to localize on the nascent centriole from metaphase onwards while PLP remains on the older centriole.

Using 3D-SIM, we are able to determine the spatio-temporal arrangement of key centriolar and centrosomal markers on *Drosophila* neuroblast centrosomes. In the future, we will also utilize 3D-SIM to analyze mutants affecting centrosome asymmetry to gain further insight into the architecture of Drosophila neuroblast centrosomes.

297B

Relationship between localization and function of the Chromosomal Passenger Complex in *Drosophila melanogaster* oocytes. Rachel A. Battaglia, Sarah J. Radford, Kim S. McKim. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08854.

In female meiosis of many animals, spindle assembly occurs without centrosomes, the microtubule organizing centers of the cell. This deviation from the centrosome-directed spindle assembly may be more error prone and can create problems during chromosome segregation. Errors in chromosome segregation are the leading cause of miscarriages and also lead to genetic diseases such as Down

syndrome. In Drosophila oocytes, the chromosomal passenger complex (CPC), which consists of INCENP, Aurora B kinase, Survivin, and Borealin, is required for chromosome-based spindle assembly and bi-orientation of centromeres. During prometaphase in oocytes, the CPC localizes with central spindle microtubules in a ring around the chromosomes and also transiently to centromeres. The mechanism of CPC function during spindle assembly is not known, but we hypothesize that the localization of the CPC is important. We will test whether centromere-localized and/or central spindle-localized CPC provide the functions of the CPC in oocytes by creating RNAi-resistant mutant Incenp transgenes that manipulate the localization of the CPC. We will force the CPC to localize to either centromeres, kinetochores, chromatin or microtubules. Conversely, we will prevent CPC localization to either the centromeres or microtubules. If CPC localization to chromatin or centromeres is not required for bipolar spindle formation then when the CPC is unable to localize to centromeres, a spindle will form, but it will have bi-orientation defects. However, if the CPC must first localize to the centromeres before it can transition to the central spindle, this mutant will fail to assemble a spindle. Preliminary data show that a wildtype RNAi-resistant Incenp transgene can rescue spindle assembly in an Incenp RNAi background. Deletion of the N-terminus cannot rescue spindle assembly, and the mutant protein localizes uniformly to the chromatin. Perhaps this uniform localization of Incenp on the chromatin impedes an increased concentration of Incenp at the centromeres, which may be required for spindle assembly to occur normally. Further observation of the effect on spindle assembly and bi-orientation by these mutants will elucidate the CPC's mechanism in meiosis.

298C

Spindle Matrix Formation is Required for Cell Cycle Progression. Jorgen Johansen, Changfu Yao, Chao Wang, Yeran Li, Jack Girton, Kristen Johansen. Biochem, Biophys & Molec Biol, Iowa State Univ, Ames, IA.

Although studies have identified critical regulatory factors acting to coordinate spindle formation and chromosome congression, how these diffusible regulatory factors are spatially organized and confined to the spindle region in the absence of a diffusion barrier after nuclear envelope breakdown (NEB) is not well understood. We identified four nuclear proteins, Skeletor, Chromator, Megator, and EAST that interact with each other and redistribute during prophase to form a dynamic, gel-like spindle matrix embedding the microtubule spindle apparatus. This matrix exists independently of microtubules and NE, and specific interactions between spindle matrix molecules are necessary for complex formation and cohesion. To address how the spindle matrix interacts with cell cycle components we have taken a live imaging approach to determine the relative timing of localization and cross-interaction of these proteins. Previously, we showed that Megator and its human homolog Tpr have an evolutionarily conserved function as spatial regulators of spindle assembly checkpoint proteins Mad2 and Mps1. Here we show that key cell cycle proteins such as Cyclin B, Polo, Ran, 14-3-3 and Endos also are co-localized at enriched levels within the spindle matrix during mitosis independent of microtubules. Furthermore, prevention of spindle matrix formation by injection of function blocking antibody to Chromator results in cell cycle arrest prior to NEB phenocopying the triple RNAi knockdown of Cyclins A, B, and B3. Interestingly, in such embryos the dynamic relocation of Polo and Cyclin B to the nuclear rim and kinetochores is abrogated and Polo is not imported into the nucleus. This is in contrast to colchicine-arrested embryos where wild-type dynamics of these proteins are maintained. Furthermore, we show that the spindle matrix prevents Pdi-GFP-marked vesicular membranes from entering the nuclear space after NEB although they are permeable to microtubules. These studies promise to provide a mechanistic framework for understanding how cell cycle factors are physically confined and organized in the spindle region, allowing for spatial and temporal integration of signaling events leading to mitotic progression and chromosome segregation.

299A

Cooperation between the kinesin motors KLP61F, Subito, and NCD promotes spindle and chromosome organization in *Drosophila* oocytes. Sarah J. Radford[1], Allysa Marie M. Go[1,2], Kim S. McKim[1,2]. 1) Waksman Institute, Rutgers University, Piscataway, NJ; 2) Department of Genetics, Rutgers University, Piscataway, NJ.

During cell division, chromosomes interact with a bipolar array of microtubules that constitute the spindle to direct their segregation. In the oocytes of most animals, including *Drosophila* and humans, the spindle is assembled in the absence of the microtubule-organizing centers called centrosomes. Without the organization provided by centrosomes, we have hypothesized that acentrosomal spindle organization relies on the bundling of microtubules by kinesin motor proteins. It has been known for many years that two microtubule-bundling kinesins – the plus-end directed kinesin-6 Subito and the minus-end directed kinesin-14 NCD – are required during oocyte spindle assembly. How these microtubule-bundling activities cooperate, and whether additional microtubule-bundling kinesins function in oocytes is not known. The most prominent microtubule-bundling kinesin in centrosomal cells is the plus-end directed kinesin-5 KLP61F, but study of the role of KLP61F in oocytes was precluded by the lethality of*Klp61F* mutants. To overcome this, we used RNAi constructs from the Transgenic RNAi Project to knock down *Klp61F* expression during meiosis in *Drosophila* oocytes. We found that loss of KLP61F leads to a distinct asymmetry of the acentrosomal spindle with one half spindle markedly weaker than the other. This correlates with an asymmetric distribution of centromeres such that fewer centromeres are oriented towards the weaker spindle pole. We are currently investigating whether factors present at acentrosomal spindle poles play a role in generating this asymmetry. We also found that loss of KLP61F frequently leads to a dispersal of chromosomes throughout the entire volume of the oocyte. This phenotype is suppressed by loss of NCD, but enhanced by loss of Subito. These results suggest a model in which chromosomes are maintained at the center of a symmetric bipolar spindle through a balance of forces. The plus-end-directed kinesins KLP61F and Subito are partially redundant and antagonistic to the minus-end-directed kinesin NCD, resulting in proper spindle and chromosome organization in acentrosomal oocytes.

300B

The roles of the Doublefault protein in chromosome segregation and cytokinesis. Stefano Sechi, Anna Frappaolo, Giorgio Belloni, Roberto Piergentili, **Maria Grazia Giansanti**. IBPM, Consiglio Nazionale delle Ricerche, Rome, Italy.

We have isolated a novel male sterile mutation affecting both chromosome segregation and cytokinesis that failed to complement the P-induced mutant d*oublefault (dbf),* previously mapped in 32A2. DNA sequencing revealed that our (dbf) allele caused a premature stop codon in the annotated *CG17098 Drosophila* gene. Based on these results dbf encodes a predicted 73kDa polypeptide, containing a C2H2-like zinc finger domain involved in nucleic acid binding. During meiotic division *dbf* affected spindle morphology, chromosome alignment and segregation. Moreover *dbf* mutants were unable to assemble both the central spindle and the contractile ring during telophase. Imaging of spermatocytes expressing a GFP-tagged protein revealed that Dbf was enriched on microtubules during prophase and accumulated around the spindle envelope and the spindle poles during meiosis. Interestingly dbf mutations disrupted the localization of RanGAP a protein involved in the establishment of a RanGTP gradient across the nuclear envelope and in the nucleocytoplasmic transport. *dbf* mutations also abolished the localization of the Chromosomal passenger (CPC) proteins at both the kinetochores and the spindle midzone. Our results suggest that Dbf might be involved in Ran-driven RNA transport and RNA-mediated recruitment of the CPC at the kinetochores required for spindle dynamics and cytokinesis.

301C

Myosin dynamics during asymmetric stem cell division. Anna Tsankova, Clemens Cabernard. Biozentrum, University of Basel, Klingelbergstrasse 50-70, 4056 Basel, Switzerland.

Drosophila neuroblasts are intrinsically polarized stem cells in the developing central brain of the fruit fly, which divide asymmetrically to generate a self-renewed stem cell and a differentiated sibling. The correct positioning of the cleavage furrow is a requirement for the correct segregation of cell fate determinants, ensuring different sibling cell fate. In neuroblasts, cleavage furrow positioning is controlled by a novel polarity-dependent pathway. These polarity cues control the asymmetric localization of the cleavage furrow component Myosin. For instance, Myosin is first depleted from the apical cortex. Later on Myosin also clears from the basal cortex and subsequently becomes restricted to the cleavage furrow by late telophase. The cellular and molecular mechanisms of asymmetric Myosin localization remain elusive. We are applying high-resolution live imaging, photoconversion experiments and FRAP to investigate the dynamics of Myosin relocalization in third instar larval neuroblasts. For instance, photoconversion experiments revealed that Myosin molecules reach the cleavage furrow via cortical flow. Our experiments thus suggest that the cleavage furrow is not established by de novo Myosin filament assembly but through redistribution of the cortical Myosin pool. Myosin is activated by phosphorylation and we are interested in the spatial and temporal activation profile of Myosin during ACD. Our immunohistochemistry results show that during early metaphase phospho-Myosin is enriched at the apical cortex. In a kinase candidate screen we found that Protein Kinase N (PKN) is, similar to phosphorylated Myosin, enriched at the apical neuroblast cortex. Our preliminary results show that this PKN asymmetry is established during early prophase and is regulated by the polarity dependent pathway. Furthermore, PKN mutants show reduced Myosin phosphorylation during metaphase and loss of physical asymmetry. Based on these results, we conclude that PKN is a component of the polarity-dependent cleavage furrow positioning pathway, instrumental in establishing sibling cell size asymmetry.

302A

snoRNAs are a novel class of biologically relevant Myc targets. Peter Gallant[1,2], Eva Herter[1,2], Maria Stauch[1,2], Maria Gallant[1,2], Elmar Wolf[1,2], Thomas Raabe[3]. 1) Biochemistry & Molecular Biology, University Wuerzburg, Wuerzburg, Germany; 2) Comprehensive Cancer Center Mainfranken, University Wuerzburg, Wuerzburg, Germany; 3) Institute for Medical Radiation and Cell Research, University Wuerzburg, Wuerzburg, Germany.

Myc proteins are essential regulators of animal growth during normal development, and their deregulation is one of the main driving factors of human malignancies. They function as transcription factors that control many growth- and proliferation-associated genes, and in some contexts contribute to global gene regulation. Here, we combine ChIPseq and RNAseq approaches in *Drosophila* S2 cells to identify a core set of less than 500 Myc target genes, whose salient function resides in the control of ribosome biogenesis. Amongst these genes we find the non-coding snoRNA genes as a large novel class of Myc targets. All assayed snoRNAs were found to be affected by Myc, and many of them are subject to direct transcriptional activation by Myc, both in *Drosophila* and in vertebrates. The loss of snoRNAs impairs growth during normal development, whereas their overexpression increases tumor mass in a model for neuronal tumors. In combination with recent observations of snoRNA involvment in human cancer, this suggests that some of Myc's transforming effects are mediated by this class of non-coding transcripts.

303B

Mitochondrial Growth & Dynamics During *Drosophila melanogaster* Oogenesis. Jasmin Imran Alsous[1], Matheus Viana[2], Susanne Rafelski[2], Stanislav Shvartsman[1]. 1) Princeton University , Princeton , NJ; 2) University of California Irvine, Irvine, CA.

How a cell regulates the size and morphology of its organelles during growth remains an open question. Mitochondria are major organelles that supply the cell with energy, and are central to most cellular functions. During oogenesis, mitochondria are the most abundant organelles in the oocyte, and are its primary source of ATP. Mitochondria are maternally inherited; their presence in the correct amount and location is critical for the successful maturation of the oocyte, and future embryo. My research focuses on understanding how the *Drosophila* egg chamber acquires its mitochondrial content, and regulates its size, location and function at various stages of its development. During oogenesis, the egg chamber experiences a massive and rapid increase in volume. The oocyte and the nurse cells, despite being germline cells, grow at different rates, and have vastly different functions within the egg chamber: the

nurse cells, with large polyploidy nuclei, are biosynthetically active, and 'nurse' the transcriptionally, and synthetically quiescent oocyte. Volumetric measurements of 3D reconstructed follicles show that nurse cells, like most somatic cells, have a constant nucleus-to-cell volume ratio throughout oogenesis. The oocyte, however, with its inactive nucleus, has a sharply decreasing karyoplasmic ratio. Nonetheless, these cells' mitochondrial volume is roughly equal and follows the same increasing trend until nurse cell dumping. This result is surprising and has two implications. First, the nurse cells' and oocytes' different functions throughout oogenesis, and their perceived energetic requirements, are not reflected in their mitochondrial content. Second, the cells' karyoplasmic ratios on the one hand, and their roughly equal previtellogenic cytoplasmic content on the other, suggests that the cells' mitochondrial content is regulated independently of their nuclear size, and is more tightly linked to their cytoplasmic content. The egg chamber is therefore not only a relevant system for studying mitochondrial growth and dynamics throughout oogenesis, but is also an excellent model for understanding how a system-level control of organelle and cell size is achieved in a rapidly growing and multi-cellular chamber. .

304C

Conditional screen for growth mutants identifies two negative regulators of Hedgehog signaling with distinct overgrowth phenotypes. Jacob Kagey, Frank Adamini, Erik Coopes, Shannon Moore, Jordan Stewart. Biology, University of Detroit Mercy, Detroit, MI.

An EMS screen was conducted on chromosome 2R using Flp/FRT in the developing eye. This screen was conducted in a genetic background of blocked apoptosis. Growth mutants identified in the screen were organized into nine different overgrowth phenotypic groups. Once categorized individual mutants were mapped using deficiency mapping and complementation. In mapping mutants to individual genes, we identified two genes, *Patched* (*Ptc*) and *Costa* (*Cos2*) that are both negative regulators of the Hedgehog (Hh) signaling pathway. Both mutations lead to dramatic eye and wing mosaic tissue overgrowth, which is conditional upon a block in apoptosis. At the molecular level both mutations lead to an autonomous increase in Hh signaling coupled with a non-autonomous increase in the expression of DIAP1. Despite both genes functioning to negatively regulate Hh signaling we identified a number of differences between the mutant phenotypes in both the mosaic eyes and wings. *Ptc* mutant eyes demonstrated non-autonomous overgrowth and *Cos2* depicted both mutant and wild type eye tissue overgrowth. *Ptc* mosaic larval wing discs grow significantly larger than *Cos2* larval mosaic wing discs, and these wing phenotypes differ on the dependence of blocked apoptosis. Understanding how negative regulators of the same molecular pathways can lead to distinct mutant phenotypes will be essential in understanding how the Hh pathway functions in normal development and can drive tissue overgrowth when mutant.

305A

Context dependent regulation of proliferation and cellular growth by Hippo-Yorkie signaling. Zhiqiang Shu, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL 32306.

Differentially regulated cell division and proliferation is a conserved strategy to maintain tissue homeostasis in metazoans. During development, cells in different cellular contexts show different proliferation and growth rate. How this context dependent proliferation is regulated remains largely unclear. Here we address this puzzle by introducing a new approach to measure *in vivo* cell proliferation rate. Through overexpression of fizzy-related (fzr), we can induce mitotic cells into endoreplication and cell proliferation rates in different cells can be compared for their nuclear size differences. Using this approach, we investigate how Hippo signaling, a central tissue growth regulator, regulates cell proliferation differently in distinct regions of the *Drosophila* wing imaginal disc. Overexpression of Hippo signaling effector Yorkie (Yki) promotes proliferation by accelerating cell mitotic cycle. Co-expression of Yki and Fzr in the wing disc shows increased nuclear size. Interestingly, this phenotype is much more prominent in the hinge and notum regions of the wing disc. In contrast, cells in the pouch region have only slightly increased nuclear sizes. This regional difference in cell growth appears to be related to differential regulation of a Hippo pathway target, *cyclin E* (*cycE*), between the pouch and hinge area. We find that Yki up-regulates cycE mainly in the pouch region, and that high levels of cycE impedes fzr-induced endoreplication. We also find that this regional growth regulation by Yki results from differential expression of its transcriptional partner Scalloped (Sd), which is expressed primarily in the pouch region, with the highest concentration at the dorsal/ventral boundary of the wing disc. Genetic interaction results further demonstrate that Sd is required for Yki to impede endoreplication in the wing pouch. Our results altogether reveal a mechanism of context dependent regulation of proliferation by Hippo signaling, which in turn controls differential proliferation and maintains homeostatic tissue growth in the *Drosophila*wing disc.

306B

Acinus Links Autophagy and Hippo Signaling. Lauren Tyra, Nilay Nandi, Helmut Krämer. Neuroscience, UT Southwestern Medical Center, Dallas, TX.

Drosophila Acinus is a highly conserved protein implicated in multiple critical cellular processes, including autophagy [1,2], endocytic trafficking [1], RNA splicing [3, 4], and cell death [5]. In order to uncover the mechanism by which Acinus functions in these diverse processes, we took a genome-wide screening approach to identify novel interactors of Acinus using a classic enhancer/suppressor screen. GMR-Gal4 mediated over-expression of Acinus in the eye causes an autophagy-dependent rough eye. This phenotype is dominantly suppressed by loss of one copy of multiple members of the Hippo pathway. Hippo signaling is an important repressor the transcriptional co-activator Yorkie, which regulates cell growth, proliferation, survival, and cancer through gene expression [6]. A complex, yet poorly understood connection between autophagy and Hippo signaling has been shown in *Drosophila* [7]; however a clear mechanism to connect these two critical processes remains unknown. To investigate how Acinus interacts with Hippo signaling, we have tested Acinus gain- and loss-of-function for known Hippo-related phenotypes. RNAi knock-down of Acinus in the wing causes

increased expression of the*expanded, four-jointed*, and *thread (diap1) lacZ* reporters, which are used to measure transcriptional output of Hippo signaling. Similar to other known negative regulators of Hippo signaling, knock-down of Acinus by two independent RNAi lines using the Engrailed-Gal4 (posterior wing compartment) or Ms1096-Gal4 (wing pouch) drivers causes wing over-growth. The converse is also true; over-expression of Acinus in the wing causes small wings. These data indicate that Acinus is a negative regulator of the transcriptional output of the Hippo signaling cascade. Elucidating the mechanism by which Acinus is repressing transcriptional targets of Hippo signaling will provide valuable insight into its function in cellular processes critical for developmental cell growth, as well as cancer.

1. Haberman 2010, PMID: 20504956.
2. Nandi 2014, in press at JCB.
3. Hayashi 2014, PMID: 25081352.
4. Malone 2014, PMID: 25104425.
5. Sahara 1999, PMID: 10490026.
6. Staley and Irvine 2012, PMID: 22174083.
7. Perez 2014, PMID 25174403

307C

Following dedifferentiation in cells with inactivated Rb and Hippo pathways. Battuya Bayarmagnai, Maxim V. Frolov. Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, 900 S Ashland Avenue, Chicago, IL 60607.

Cell cycle exit and differentiation are integral to the proper development of an organ. The Retinoblastoma (Rb) and Hippo tumor suppressor pathways regulate cell cycle and organ growth, respectively. Our previous work unveiled an unexpected finding that eye imaginal disc cells with inactivated Rbf and Hippo pathways lose the expression of photoreceptor markers. These double mutant cells are not being eliminated in a caspase-dependent manner, since overexpression of p35, which blocks caspase activity, did not rescue this phenotype. Cell marker analysis done so far supports the hypothesis that the double mutant cells fail to maintain their differentiated state, and thus undergo dedifferentiation. In order to precisely determine the new identity of the double mutant cells, we built a labeling system that allows tracing of these cells. We employed a combination of UAS/Gal4 and mFlp5/mFRT71 systems to label photoreceptor cells with the expression of the *lacZ* gene. LacZ expression is induced in mature photoreceptors. However, its continued expression becomes independent of the developmental stage or cell identity, allowing us to follow the double mutant cells even after they lose the expression of their differentiation markers and subsequently, their identity. Using this system, we can clearly visualize the LacZ-positive double mutant cells, and have conclusively demonstrated that the double mutant cells persist in the eye discs, but lose the photoreceptor markers. Furthermore, this new system will allow us to determine the new identity of these cells and study the mechanism by which cells maintain their differentiated state. .

308A

Quantitative analysis of EGFR-regulated size control in the *Drosophila* epidermis. Pavel A Brodskiy[1], Jochen Kursawe[2], Cody Narciso[1], Ruth Baker[2], Alexander Fletcher[2], Jeremiah J. Zartman[1]. 1) Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN; 2) Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, United Kingdom.

The control of size and shape of organ compartments is an outstanding question in the field of developmental biology. While much is known about how signaling pathways such as the epidermal growth factor receptor (EGFR) cascade regulate growth, proliferation and apoptosis (programmed cell death), much is still unknown about how relative signaling levels and topological inputs translate into determination of tissue properties. To address this question, we used high-resolution confocal microscopy and an in-house image-processing pipeline to quantify cell topology, network statistics, and gene expression in the *Drosophila* embryonic epidermis, in order to better understand the patterning and size control functions of EGFR signaling. Due to the transient nature of apoptosis, few attempts have been made to quantitatively characterize the cellular processes that regulate it or the effects of apoptosis on tissue morphology. We have defined a set of topological and morphometric summary statistics for stage 8-13 *Drosophila* embryos to inform a computational model of the epidermis that includes intercellular signaling and cell mechanics. The resulting integrated model incorporates both a vertex-based description of cellular mechanics and an independent reaction-diffusion description of the EGFR gradient in the developing epithelium. Genetic perturbations using the GAL4-UAS system for proliferation and EGFR signaling were used to gain insight into the strategies employed during development to generate tissues of the correct size and shape, providing evidence that epithelial topology is an input into cell fate decisions. This approach highlights how biochemical signaling and tissue mechanics inputs are integrated to regulate tissue size homeostasis.

309B

Motor neuron dependent regulation of myoblast proliferation uses the EGF signaling pathway. Joyce Fernandes, Kumar Vishal. Biology Dept, Miami Univ, Oxford, OH.

The indirect flight muscles of the *Drosophila* thorax are some of the largest muscles in the fly. They develop during the first day of metamorphosis, from a starting pool of myoblasts that increases from a few hundred, to about 3000 per fiber. Denervation studies have shown that motor neurons are needed for a surge in proliferation seen during a time when myoblast fusion is at its peak. To investigate motor-neuron regulation of proliferation, the EGF signaling pathway was examined. OK371Gal4 was used to target UAS-Vein RNAi and UAS Spitz RNAi to motor neurons during the first 24 hours of metamorphosis. Targeted expression of Vein RNAi resulted in less than

the normal complement of 6 DLM fibers in 60% of experimental animals. Fewer myonuclei were observed in experimental animals as compared to controls, and accounted for a 27% reduction ($p<0.05$). Targeted expression of Spitz RNAi has less severe effects on DLM fiber number, although the number of myonuclei was reduced by a comparable amount (23%). The reduced number of myonulcei was determined to be due to defects in myoblast proliferation during the myogenic period in the pupa. A 20% reduction in proliferation was sufficient to cause the observed defects in the adult. In both sets of manipulations, flight ability was significantly impaired. When secretion was blocked in motor neurons, using UAS-DN Shibire, or UAS-DN-Rab5, the muscle pattern was more severely affected than what is observed when EGF ligands are individually manipulated. These results support a role for motor neurons in regulating myoblast proliferation through cell communication mediated by the EGF signaling pathway.

310C

Insulin signaling acts downstream of ecdysone to mediate intra-organ growth coordination in *Drosophila* larvae. Rewatee Gokhale[1], Takashi Hayashi[2], Christopher Mirque[3], Alexander Shingleton[3,4]. 1) Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824; 2) Division of Developmental Genetics, National Institute of Genetics, Mishima, Shizouka 411-8540, Japan; 3) Department of Biology, Lake Forest College, Lake Forest, IL 60045; 4) Department of Zoology, Michigan State University, East Lansing, MI 48824.

Regulation of final organ size is a complex developmental process and involves the integration of systemic and organ-specific processes. Previously, we have shown that in developing *Drosophila,*perturbing the growth of one imaginal disc retards growth of other discs and delays development. Excitingly, this inter-organ growth coordination can be disrupted by exogenous application of 20E, suggesting that low levels of 20E *in vivo* are responsible for coordinating growth between organs. Here we test the hypothesis that 20E signaling is also involved in coordinating growth within an organ. Using mitotic recombination in combination with a *Minute* allele, we generated larvae in which the two compartments of the wing imaginal disc have ostensibly different growth rates (wild-type or growth-perturbed). We find that there is tightly coordinated growth between wild-type and growth-perturbed compartments, which can be disrupted through application of exogenous 20E. This suggests a universal role for ecdysone signaling in coordinating growth both between and within organs. We further explore the mechanisms downstream of ecdysone signaling that coordinate growth. Our data indicate that changes in insulin-signaling also disrupt growth coordination between wild-type and growth-perturbed developmental compartments. Importantly, 20E fails to disrupt growth coordination in discs with autonomous down-regulation of insulin-signaling, suggesting that insulin-signaling is downstream of ecdysone in regulating growth coordination. Our research therefore reveals a novel role for disc-autonomous insulin signaling in the context of growth coordination. .

311A

Junctional tumor suppressor Dlg interacts with 14-3-3 proteins to control planar alignment of the mitotic spindle in *Drosophila* epithelia. Yu-ichiro Nakajima[1], Zachary Lee[1], Matthew C. Gibson[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, KS.

The proper orientation of cell division is essential for a variety of developmental events, including tissue morphogenesis and cell fate decisions. During symmetric division of epithelial cells, planar alignment of the mitotic spindle ensures the maintenance of polarized tissue architecture, and spindle misorientation can disrupt junctional integrity, leading to cell delamination and apoptosis. While prior *in vitro* studies have implicated polarity determinants in the control of spindle orientation, the molecular mechanisms regulating epithelial planar spindle orientation *in vivo* remain largely unknown. We previously reported that the junction-localized neoplastic tumor suppressors Scribble (Scrib) and Discs Large (Dlg) control planar spindle alignment in the *Drosophila* wing disc epithelium. In contrast to current models where a Partner of Inscuteable (Pins)-Dlg interaction plays a key role in neuroblast spindle orientation, here we show that Pins is dispensable for planar spindle orientation in the wing disc. To identify proteins required for junction-mediated spindle orientation in proliferating epithelia, we performed MudPIT proteomic analysis and identified 14-3-3 proteins (14-3-3e /14-3-3z) as a potential molecular link between polarized junctional cues and the mitotic apparatus. The distribution of 14-3-3 proteins during epithelial mitosis is consistent with localization to the mitotic spindle and the cell cortex. Loss of 14-3-3 proteins in the wing disc cause abnormal spindle orientations and increased cell death, and biochemical analyses indicate that 14-3-3 proteins may function together with Dlg and the spindle regulator Mud. Taken together, these data suggest a novel molecular mechanism that couples junctional cues to the mitotic spindle during planar spindle alignment in proliferating epithelia.

312B

Non-cell autonomous tumor progression by cellular senescence. Mai Nakamura[1], Shizue Ohsawa[1], Tatsushi Igaki[1,2]. 1) Kyoto University, Kyoto, Japan; 2) PRESTO, JST, Japan.

Cell-cell communications in tumor microenvironment play important roles in cancer progression. Here, we show in *Drosophila* imaginal discs that Ras activation and mitochondrial dysfunction (Ras^{V12}/$mito^{-/-}$), frequent alterations in human cancers, cause cellular senescence and senescence associated secretory phenotype (SASP), which leads to overgrowth of neighboring tissue. Ras^{V12}-expressing cells express several hallmarks of cellular senescence such as elevation of senescence-ssociated ß-galactosidase (SA-ß-gal) activity, upregulation of the Cdk inhibitor Dacapo, heterochromatinization, and cellular hypertrophy. Strikingly, defects in mitochondrial function cause Ras-activated cells to undergo DNA damage response, cell cycle arrest, and thereby induce SASP, exhibiting full aspects of cellular senescence. Mechanistically, mitochondrial defects in conjunction with Ras^{V12} cause production of reactive oxygen species, downregulation of CycE activity, and activation of p53, which cooperate together to trigger a cell cycle arrest-JNK feedback loop that amplifies JNK activation, leading to secretion of the inflammatory cytokine Upd. Our data suggest that

mitochondrial defects promote Ras-induced cellular senescence and thereby contribute to non-cell autonomous tumor progression through SASP.

313C

Genetic control of tissue specific growth in the larval trachea of *Drosophila*. Erin Suderman, Alex Matlock, Collin Clay, Robert Ward. Molecular Biosciences, Univ. Kansas, Lawrence, KS.

In most organisms, different tissues and organs grow at different rates relative to each other, suggesting growth mechanisms that act tissue specifically. The mechanisms of tissue specific growth are less well understood than those governing the growth of an entire organism. To gain a better understanding of these tissue specific growth mechanisms we have been characterizing mutations that specifically alter growth of the larval trachea. Larval trachea growth is well suited for these studies since the trachea shows allometric growth during the larval stages, it can be imaged and measured in living animals, and gene expression can be specifically altered in the trachea using *breathless-GAL4*. Importantly, we and others have identified mutations in genes whose mutant phenotypes suggest that they normal regulate tissue-specific growth in the larval trachea. For example, animals with mutations in *uninflatable (uif)* and *Matrix metalloproteinase 1 (Mmp1)* have larval tracheae that are roughly half the relative size of those in wild type animals. Through EMS and *P*-element screens of larval lethal mutations, we have obtained 8 additional tracheal growth mutations (representing 6 complementation groups) showing either reduced or enhanced relative tracheal growth during larval stages. One *P*-element insertion is in the gene *jitterbug* (*jbug*), which encodes a filamin repeat protein. We have completed whole genome sequencing of the 7 EMS mutations and are conducting deficiency complementation analyses and tracheal-specific RNAi expression of candidate genes. We will present the basic characterization of these mutations and our efforts to clone these genes.

314A

The *big bang gene*: a novel interactor of *crumbs*. Giorgos Tsoumpekos, Linda Nementscke, Elisabeth Knust. Max Planck Institute (MPI-CBG) , Dresden, Germany.

One key regulator of epithelial polarity is the transmembrane protein Crumbs (Crb). So far, little is known about the function of its large extracellular domain. In *Drosophila*, it has been implicated in size control during head development.The aim of this study was to find new direct/indirect interactors of the extracellular domain of Crb. An enhancer/suppressor screen was previously conducted in our lab, based on the modification of a dominant wing phenotype induced upon overexpression of the extracellular domain of Crb. One of the enhancers identified is a gene called *big bang* (*bbg*). Knocking down *bbg* remarkably enhances the small wing phenotype obtained upon overexpression of the extracellular domain of Crb. Wing size is not altered upon knocking down *bbg* alone. *bbg* encodes a PDZ domain protein, which is expressed in the wing pouch of larval imaginal discs. As previously reported, flies homozygous mutant for *bbg* are viable and fertile. Most importantly, in *bbg* mutant wing discs Crb's and DPatj's localization is affected, while localization of the septate junction marker Discs Large remains unaltered. A polyclonal antibody against Bbg was generated and the protein is localized in the apical cortex in the epithelium of the wing discs. Bbg is highly expressed in the presumptive margin area and is partially colocalized with Crb, especially in the edges of the hexagonal structured cells. Mutant clones for both proteins (Crb & Bbg) were generated in wing discs and the absence of Crb, leads to reduction of Bbg from the apical membrane and *vica versa*. Immunoprecipitation experiments with the Bbg antibody, using *Drosophila* tissue lysates, showed interaction with Crb but not with Sdt and DPatJ, the main components of the Crb complex. Open questions are still remaining to this project. What is the function of *bbg* alone in epithelial polarity and cell morphogenesis? What is the link between Crb, Bbg and growth? Current work aims to understand how Crb and Bbg interact at the cellular/molecular level.

315B

Functions of Yorkie and the polarity proteins at the cell cortex. Jiajie Xu[1], Pamela Vanderzalm[2], Richard Fehon[1]. 1) University of Chicago, Chicago, IL; 2) John Carroll University, University Heights, OH.

The function of Yorkie (Yki) as a transcriptional co-activator that acts downstream of the Hippo pathway have been well studied. Recent studies suggest that the apical junctional region (AJR) is an important site of Hippo pathway regulation. Intriguingly, overexpression of a transcriptionally inactive form or a membrane tethered form of Yki in wing imaginal discs causes increased abundance of Hippo pathway components at the AJR, indicating that this effect is due to Yki functioning at the cell cortex rather than in the nucleus. Moreover, co-transfection of membrane tethered Yki with Hippo pathway components in S2 cells resulted in membrane targeting of these pathway components. Taken together, these results suggest a potential non-transcriptional, cytoplasmic function of Yki in organizing the Hippo pathway components at the AJR. Recent work also show that perturbation of polarity can cause growth defects through inactivation of the Hippo pathway. Conversely, inactivation of the Hippo pathway affects apical-basal polarity. In addition, members of the Hippo pathway co-localize with apical polarity proteins at the AJR. These observations suggest functional interactions between that Hippo pathway and apical polarity proteins. We have explored the relationship between polarity and the Hippo pathway using two approaches. In the first, we designed a genetic interaction assay to identify polarity proteins that interact with the Hippo pathway. Our results indicate that knocking down Bazooka/Par-3 (Baz) or aPKC, two apical polarity proteins, strongly enhances the overgrowth effects of overexpression of Yki. These data raise the possibility that Baz and aPKC directly regulate Yki activity. In the second approach, we have examined a possible cytoplasmic function of Yki in regulating the stability or localization of apical polarity components. Expression of a transcriptionally dead form of Yki results in increased abundance of Baz and aPKC apically, suggesting that Yki interacts with one or more polarity components in the cell cortex. Future experiments will examine interactions between Yki and Baz/aPKC and the effect of these interactions on protein stability and activity.

316C

The *Drosophila* tumor suppressor Tid glycosylates TNFR to control Hippo signaling. Geert de Vreede[1,2], Holly Morrison[1], Ditte Andersen[3], Julien Colombani[3], Pierre Leopold[3], David Bilder[1]. 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Developmental Biology, Utrecht University, Utrecht, The Netherlands; 3) University of Nice-Sophia Antipolis, CNRS, INSERM, Institute of Biology Valrose, Parc Valrose, Nice, France.

Drosophila tumor suppressor genes have been instrumental in studies of organ size, but our understanding of the molecular mechanisms controlling tissue architecture and growth remains incomplete. Here we report that phenotypes seen in *tumorous imaginal discs (tid)*, previously attributed to inactivation of a DnaJ-like chaperone, are in fact due to mutations in the N-linked glycosylation pathway component ALG3. *Tid/alg3* mutant imaginal discs show moderate dysplastic overgrowth and architecture defects that share characteristics of both 'neoplastic' and 'hyperplastic' tissues. While epithelial polarity remains intact, pro-growth Hippo signaling is upregulated, and blocking Yki activity is sufficient to suppress tumorous overgrowth. Elevated Yki activity results from aberrant JNK signaling driven by the TNF receptor Grindelwald (Grnd), recently described by the Leopold lab; N-linked glycosylation is required to prevent ectopic Grnd activation. Grnd mislocalization is seen in both N-glycosylation defective mutants as well as neoplastic mutants, where it is also required for overgrowth. Our results suggest that altered TNFR trafficking, dependent on N-glycosylation, can drive transformation of multiple*Drosophila* tumor types.

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317A

Time series Profiling identifies co-regulated genes involved in RasV12- immortalization of muscle precursors. Mary-Lee Dequeant[1], D. Fagegaltier[2], Y. Hu[1], A. Simcox[3], G. Hannon[2], Norbert Perrimon[1]. 1) Harvard Medical School, Boston, MA; 2) Cold Spring Harbor Laboratory, NY; 3) Ohio State University, OH.

Unbiased time series transcriptomics is a powerful approach to characterize dynamic biological phenomenon. Here we apply this approach to characterize the derivation of *Drosophila* cell lines. Usually cumbersome and difficult, this process becomes highly efficient when starting from *Drosophila* embryonic primary cultures expressing RasV12. However, some key questions remain: what are the origins of the cells and their properties? What regulation is involved during RasV12 immortalization?

To characterize these lines and the sequential events that drive them towards an immortalized state**,** we generated transcriptional RNA-Seq time series during the immortalization of five independent primary cultures over six months. Differential expression analysis followed by gene ontology analysis revealed that stabilization of the cultures over time is associated with up-regulation of genes involved in cell cycle and epigenetic regulation. Furthermore, the data suggest an enrichment of the cultures in neurogenic and myogenic tissue types. In particular, we were able to identify a transcriptional signature reminiscent of Adult Muscle Precursor cells (AMPs). Taking advantage of the high information content of time series profiling, we performed a correlation analysis using the Twist transcription factor expression profile as a bait. As a result, in addition to known markers of AMPs, we identified candidate genes for roles in the proliferation and maintenance of AMPs. We are currently validating some of the candidates *in vivo*. Furthermore, consistent with the origins of AMPs, the cells can be differentiated into muscle cells, suggesting that RasV12 immortalization is reversible. In summary, this study identifies regulators of muscle proliferative progenitors and opens new avenues for the derivation of progenitor cell lines.

318B

Nucleoporins Nup98-96 are required for normal tissue growth and proper cell cycle regulation. Kiriaki Kanakousaki, Olga Grushko, Laura Buttitta. Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

Nucleoporins (Nups) are essential components of the Nuclear pore complexes (NPCs) that regulate transportation of macromolecules between nucleus and cytoplasm. However, some Nups (such as Nup98) are also found in the nucleoplasm, where they regulate gene transcription. Chromosomal translocations that result in Nup98 fusions with several other protein partners are found in many hematological malignancies. However, it is not yet understood how Nup98 function is related to the disease or how disruption of the Nup98-96 locus upon translocation (which produces both Nup98 and Nup96 proteins) contributes to cancer formation.

We discovered that reduction of both Nup98 and Nup96 via RNAi results in dramatic cell cycle de-regulation. When Nup98-96 is compromised, wing cells contain an abnormal DNA content, consistent with an aberrant bypass of G1 cell cycle arrest and abnormal S-phase length. However, Nup98-96 deficient cells are not trapped in S-phase, as many of them are positive for the mitotic marker, phospho-Histone3, suggesting an overall acceleration of cell cycle. However, tissue overgrowth is not observed due to a concomitant increase in apoptosis. This is accompanied by an increase in JNK activation and expression of Wingless, hallmarks of a wound response in fly wings. When apoptosis is blocked by the Caspase inhibitor p35, cell cycle de-regulation of Nup98-96-deficient cells causes tissue overgrowth. Surprisingly, when p35-rescued Nup98-96-deficient cells occupy the majority of a tissue, apoptosis becomes triggered in the neighboring wild-type cells, indicating a non-autonomous effect of compromising Nup98-96.

Together, our data indicate a significant role for Nup98-96 in cell cycle regulation and tissue growth. Identification of the mechanism underlying Nup98-96 roles in cell cycle regulation will elucidate how Nup98-96 loss contributes to cancer development.

319C

***Drosophila C-terminal Src kinase* regulates growth via Hippo signaling pathway. Hailey J. Kwon**[1], Indrayani Waghmare[1], Shilpi Verghese[1], Aditi Singh[4], Amit Singh[1,2,3], Madhuri Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Center

for Tissue Regeneration and Engineering at Dayton, Dayton, OH; 3) Premedical Programs, University of Dayton, Dayton, OH; 4) Centerville High School, Centerville, OH.

The Hippo signaling pathway is involved in regulating tissue size by inhibiting cell proliferation and promoting apoptosis. Aberrant Hippo pathway function is often detected in human cancers and correlates with poor prognosis. The *Drosophila C-terminal Src kinase* (*d-Csk*) is a genetic modifier of *warts* (*wts*), a tumor-suppressor gene in the Hippo pathway, and interacts with the Src oncogene. Reduction in *d-Csk* expression and the consequent activation of *Src* are frequently seen in several cancers including hepatocellular and colorectal tumors. Previous studies show that *d-Csk* regulates cell proliferation and tissue size during development. Given the similarity in the loss-of-function phenotypes of *d-Csk* and *wts*, we have investigated the interactions of *d-Csk* with the Hippo pathway. Here we present multiple lines of evidence suggesting that *d-Csk* regulates growth via the Hippo signaling pathway. We show that loss of dCsk caused increased Yki activity, and our genetic epistasis places dCsk downstream of Dachs. Furthermore, dCsk requires Yki for its growth regulatory functions, suggesting that dCsk is another downstream member of the network of genes that interact to regulate Wts and its effector Yki in the Hippo signaling pathway. .

320A

Impaired Hippo Signaling Promotes Rho1-JNK Dependent Growth. Xianjue Ma[1,2], Yujun Chen[2], Duojia Pan[1], Lei Xue[2]. 1) Molecular Biology & Genetics, Johns Hopkins School of Medicine, Baltimore, MD; 2) School of Life Science and Technology, Tongji University, Shanghai, China.

Both Hippo and JNK signaling have well-established roles in promoting tissue growth and give rise to tumorigenesis when dysregulated, while it remains elusive how the two pathways interact or coordinate in growth control. While Hippo pathway acts via the transcription co-activator Yki/YAP to regulate target genes expression, JNK signaling, triggered by various modulators including Rho GTPases, activates the transcription factors Jun and Fos. Here, we show that impaired Hippo signaling induces JNK activation through Rho1. Blocking Rho1-JNK signaling suppresses Yki-induced overgrowth, while ectopic Rho1 expression promotes tissue growth when apoptosis is prohibited. Furthermore, Yki directly regulates Rho1 transcription via the transcription factor Sd. Finally, we find such functions of Yki have been conserved in its mammalian ortholog YAP, which activates Sd-dependent Rho1 transcription and JNK signaling in *Drosophila*. Our results have identified a novel molecular link between the Hippo and JNK pathways, and implicated the essential role of JNK pathway in Hippo signaling related tumorigenesis.

321B

The DHHC palmitoyltransferase *approximated* regulates Fat signaling and Dachs localization. Hitoshi Matakatsu[1,2], Seth Blair[2], Richard Fehon[1]. 1) MGCB, The University of Chicago, Chicago, IL; 2) Dept. Zoology, Univ. Wisconsin-Madison, WI.

Signaling via the large protocadherin Fat (Ft) and Dachsous (Ds) is required for a variety of developmental functions in *Drosophila* and humans. Ft and, to a lesser extent, Ds suppress overgrowth of the imaginal discs by suppressing Hippo signaling pathway. Ft and Ds are also required for normal planar cell polarity (PCP) in wing, eye and abdomen. Ft signaling has been shown to be negatively regulated by the atypical myosin Dachs. However it is not clear how Ft signaling regulates growth control via Hippo signaling pathway.
Our previous work identified the Approximated (App) as an additional negative regulator of Ft signaling in growth control. We showed that App encodes a member of the DHHC family, responsible for the palmitoylation of selected cytoplasmic proteins. Mutations in core catalytic domain caused *app*-like phenotype, suggesting that App acts as palmitoyltransferase. In the absence of App function, Dachs fails to localize to the sub-apical membrane and is inactive, suggesting that App recruit Dachs at sub-apical membrane. Although this result suggests that Dachs might be a target for App-mediated palmitoylation, our data indicate that Ft is palmitoylated instead of Dachs. We are currently examining how post-traslational modification(s) regulates Ft signaling. .

322C

Ras-ERK signaling controls growth in imaginal discs and intestinal stem cells via synthesis of rRNA and tRNA. Shrivani Pirahas, Savraj S. Grewal. Clark H. Smith Brain Tumour Centre, Southern Alberta Cancer Research Institute, Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada.

Over the last two decades, genetic studies in *Drosophila* have identified many of the improtant cell signaling pathways and networks that control cell and tissue growth. One such pathway is the Ras-ERK pathway. Ras- ERK as been shown to promote cell growth and proliferation in many tissues throughout *Drosophila* development. Moreover, overactive Ras-ERK signaling is observed in many cancers and can drive tumorgenesis. An important challenge is to identify how the Ras-ERK pathway influences cellular metabolism to drive growth. Here we report on the control of both rRNA and tRNA synthesis as growth effectors of Ras-ERK signaling. We show that activation of ERK signaling pathway by overexpressing activated forms of EGFR, Ras or Raf in wing imaginal discs leads to an increase both rRNA and tRNA synthesis. Similarly, expression of oncogenic RasV12 in *Drosophila* S2 cells increases tRNA levels, while pharmacological inhibition of ERK signaling by the MEK inhibitor, U0126 leads to reduced rRNA and tRNA synthesis. RNA polymerases I (Pol I) and III (Pol III) transcribe rRNA and tRNA respectively. We previously identified the Pol I and Pol III factors, TIF-IA and Brf, as regulators of cell and tissue growth in *Drosophila*. Here we show that knockdown of either TIF-IA or Brf blocks the effects of Ras-ERK signaling on growth and proliferation in larval wing imaginal discs, adult midgut progenitor cells and adult intestinal stem cells. We are investigating how Ras-ERK signaling regulates TIF-IA- and Brf-dependent transcription. Given the importance of both rRNA and tRNA synthesis in ribosome biogenesis and mRNA translation, our studies point to control of protein synthesis as an important effector of Ras-ERK signaling in regulating growth.

323A

Apical delamination of pro-tumor cells in intrinsic tumor hotspots initiates tumorigenesis. Yoichiro Tamori[1], Emiko Suzuki[1], Wu-Min Deng[2]. 1) Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka, Japan; 2) Department of Biological Science, Florida State University, Tallahassee, FL.

Malignant tumors are caused by uncontrolled proliferation of transformed mutant cells that have lost the ability to maintain tissue integrity. Although a number of causative mutations for oncogenes or tumor-suppressor genes have been discovered, the initial steps mutant cells take to escape cellular defense mechanisms and trigger tumorigenesis remain unclear. In our analysis of conserved neoplastic tumor-suppressor genes (nTSGs) in *Drosophila* imaginal epithelia, we identified a specific region in which tumorigenesis always originates. In this "tumor hotspot," nTSG mutant cells delaminate from the apical side of the epithelia and undergo tumorigenic overgrowth. Conversely, in other regions dubbed "tumor coldspots," nTSG mutant cells are outcompeted by their wild-type neighbors, causing them to be basally extruded and undergo apoptosis. Further analysis of cytoarchitectural differences between the coldspots and hotspots revealed three intrinsic cellular structures observed specifically in the tumor hotspots: basal enrichment of microtubules, intertwining basal filopodia, and tightly laminated basement membranes. The hotspot-specific cytoarchitecture prevents the nTSG mutant cells from being extruded from the basal side and promotes their apical delamination. Given the conservation of the epithelial cytoarchitecture, tumorigenesis could be generally initiated from tumor hotspots in a similar mechanism.

324B

JNK-Yki mediated signal amplification loop promotes tumorigenesis in epithelial cells. Indrayani Waghmare[1], Shilpi Verghese[1], Austin Roebke[1], Hailey Kwon[1], Amit Singh[1,2,3], Madhuri Kango-Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Programs, University of Dayton, Dayton OH 45469; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), Department of Biology SC333, University of Dayton, 300 College.

The inter-cellular interactions via short- and long-range signaling are critical for normal development, physiological functions of cells, and maintenance of tissue homeostasis. These inter-cellular interactions are also critical for pathological processes like tumorigenesis and metastasis. To uncover the intercellular signals that promote tumorigenesis we analyzed the loss of function of *Drosophila* scribble (*scrib*$^{-}$) gene in different microenvironments. We present several novel findings that contribute significantly to our understanding of how oncogenic *Ras*^{V12} uncovers the tumorigenic potential of scrib- cells. The distinct changes in levels and localization of Wg, Dronc, JNK and Yki in growth competent scrib- cells underlie their growth potential. We found that multiple pathways (JNK, Dronc, Yki, Wg) play a tumor-promoting role, and are required for aggressive tumor growth. We demonstrate that these signals form a context-dependent signaling module wherein JNK and Yki form a positive feed-back signal amplification loop, which promotes the sustained aggressive growth of *Ras*^{V12},*scrib*$^{-}$ tumor cells. In the absence of this JNK-Yki signal amplification loop tumor growth is suppressed. scrib- can autonomously and non-autonomously induce Yki, JNK and Wg in a Yki overexpressing sensitized background. Further, we show that increased Yki activity can cause aggressive growth in *scrib*$^{-}$ cells in the absence of oncogenic *Ras* due to the establishment of the JNK-Yki mediated signal amplification loop. Lastly, oncogenic *Ras* restricts non-autonomous induction and promotes autonomous induction of Yki, JNK and Wg in *scrib*$^{-}$ cells. Oncogenic cooperation between activated *Ras* and loss of *scrib* also occurs in multiple mammalian cancer models.Overall, this study provides a strong genetic evidence for oncogenic cooperation between *scrib*$^{-}$ and *Ras*^{V12} and the signaling framework within which they cause tumorigenesis.

325C

Regulation of cell competition by the cytokine Spätzle. Lale Alpar[1], Laura Johnston[2]. 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Department of Genetics and Development, Columbia University 701 W168th Street, New York, NY.

During development, genetic differences that alter cellular growth rates or metabolic properties can arise in proliferating tissues, leaving some cells less fit than others. Such heterogeneities in fitness lead to cell competition. As a result, suboptimal cells are eliminated by apoptosis while the relatively more fit cells over-proliferate in compensation. We have identified the secreted cytokine Spätzle (Spz) as a potential activator of a signalling pathway requiring Toll-related receptors and NFκB factors that leads to the elimination of sub-optimal cells during cell competition. Spz is the activating ligand of the Toll receptor, best known for its role in the embryonic dorsal-ventral patterning and innate immunity. In both of these contexts, Spz is secreted as an inactive proprotein that is proteolytically activated via serial activation of a set of serine proteases (SPs). Using clonal analysis we show that Spz is required for cell competition, but this requirement is not specific to the competing cell populations of the wing imaginal disc. This implies that the activation of Spz, rather than its expression, is controlled locally at sites of cell competition. We find that several SPs known to regulate Spz in innate immunity or DV patterning are expressed in wing imaginal discs. Our results also indicate that some of the SPs are required in "loser" cells for their elimination. Based on these data, we postulate that Spz is a systemic sensor of fitness differences between cells. We propose that spatial and temporal control of Spz activation by local factors is a critical event in the detection of cell fitness differences, and in initiating the signalling pathway that leads to the death of "loser" cells and proliferation of "winner" cells. To test these ideas, we aim to identify 1) where Spz is produced, 2) the regulatory mechanisms controlling Spz activity, and 3) how these mechanisms are deployed during cell competition. Ultimately, we hope to understand the mechanisms by which cells perceive and respond to fitness differences within growing tissues.

326A

Analysis of Toll-related receptor function in triggering cell death during cell competition. C. Bergantinos, L. Johnston. Department of Genetics & Development, Columbia University Medical Center, 701 West 168th Street, HHSC 704, New York, NY, 10032.

Tissues that contain weak or damaged cells represent potential risks to the animal. Accordingly, the appearance of a population of sub-optimal cells in a growing tissue elicits cellular interactions that prevent their contribution to the adult. This process, called cell competition, is conserved from insects to mammals and leads to the apoptotic elimination of the relatively less fit cells in a tissue while the more fit cells proliferate more to fill the lost tissue. We recently showed that this quality-control process utilizes components of the evolutionarily ancient innate immune system, including Toll-related receptors (TRRs), the ligand Spätzle (Spz), and the NFkB factors Relish, Dif and Dorsal to trigger the elimination of the loser cells by inducing expression of the pro-apoptotic genes *hid* or *reaper*. Here we address the specific roles of four TRRs we identified that are each required to kill loser cells in Myc-induced competition. We consider two models for how they function in cell competition. In one model, we postulate that the cytokine Spz functions as the ligand for the TRRs, thereby activating NFkB factors and triggering their death. A second model posits that homophilic or heterophilic adhesive interactions mediated by the TRRs contributes to recognition and/or interactions between the winner and losers cells. We are using a combination of genetic analysis and molecular techniques to determine the exact role of the TRRs during *Myc*-induced and *Minute*-induced cell competition, how they signal to the NFkB factors, how they functionally interact and how they are regulated. These studies will pave the way to understanding how cells in growing tissues sense and respond to genetic or fitness differences in development and disease. .

327B

Defining the loser cell identity in cell competition. Iwo Kucinski, Eugenia Piddini. GURDON INSTITUTE, UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, United Kingdom.

Cell competition is a process during which a fitter cell population outcompetes a population of neighbouring weaker cells, inducing their elimination. Despite its discovery four decades ago, our knowledge of the molecular mechanisms governing cell competition is limited. We sought to identify the molecular signature of loser cells and to this aim we have analysed the transcriptional profiles of wing discs from a set of loser-associated mutations. We have compared RNAseq data from mutations in genes that are seemingly functionally unrelated, yet share the loser phenotype. As a result we have identified ~300 genes associated with the loser status - these are differentially expressed in loser cells compared to wild-type cells. GO term enrichment analysis shows the activation of signalling pathways associated with cell survival but also activation of genes that regulate the cell death response. We are currently investigating the relevance of these pathways to the physiology of loser cells.

328C

Delineating the machanism of postmitotic cell competition. Sarayu Row, Pang-Kuo Lo, Dongyu Jia, Yoichiro Tamori, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

Cell competition in multicellular organisms helps to maintain organ size and tissue integrity. This phenomenon is a cellular response to genetic mosaicism within a tissue. Aberrant but viable cells are eliminated through cell competition in epithelial tissue as 'winner' cells induce apoptosis in neighboring 'loser' cells. We recently reported that cell competition occurs in postmitotic follicular epithelia, where cell death (apoptosis) induced by a gene defect occurs only in a mosaic and not in homogeneous tissue. The detailed mechanism of postmitotic cell competition (PCC), however, remains largely unknown. To explore the biological processes involved in PCC, we screened ~3400 RNAi lines for cell competition driven apoptosis in postmitotic follicular epithelia of developing egg chambers in *Drosophila melanogaster*. We used two Gal4 drivers, Geneswitch and TARGET, to generate a mosaic and a homogeneous system of RNAi expressing cells, respectively. From the *in vivo* RNAi screen, we have identified nine genes involved in various biological processes including transcriptional regulation, protein ubiquitination and sumoylation, cellular oxidation-reduction process, regulation of signal transduction, establishment or maintenance of cell polarity and vesicle-mediated transport. Further characterization of the role of these genes in cell competition will give a comprehensive insight into the underlying mechanism of PCC in follicular epithelia.

329A

Cell shape homeostasis against differential proliferation during epithelial cell competition. Alice Tsuboi[1], Shizue Ohsawa[2], Kenji Matsuno[1], Tatsushi Igaki[2], Koichi Fujimoto[1]. 1) Dept. of Biol. Sci., Grad. Sch. of Sci., Univ. of Osaka, Osaka, Japan; 2) Grad. Sch. of Biostudies, Univ. of Kyoto, Kyoto, Japan.

Cell competition is a mechanism of tissue homeostasis against cell-cell heterogeneity, whereby apoptosis occurs into slower-dividing cells ('losers') among cells dividing more rapidly ('winners') in multicellular communities. When over-proliferating, e.g., oncogenic, mutations appear in homogeneous tissue, increased cell density raise actomyosin-dependent mechanical tension so that cells should change the cell shape or division rate to maintain the tissue shape and the cell density. However, what types of homeostasis against the differential proliferation is largely unknown. Here we combined computer simulations and genetic experiments in *Drosophila* to study how the differential growth rate disrupts the interface between two clonal cell populations with different cell division rates. When such populations were introduced into a mathematical-"cell vertex" model, which quantitatively accounts for the packing geometry of normal epithelial cells, we numerically found that the boundary of the clones was smoothly rounded. At the boundary, the slower dividing cells exhibited abnormalities not only in their shape, but also in tensile force. Consistent with the numerical predictions, the mosaic experiments in *Drosophila*wing imaginal epithelium showed that the clone interface between wild-type and Hippo pathway mutant (faster-dividing) cells was rounded and the cells around clone interface are elongated. These mathematical and experimental data indicate that the differential proliferation perturbs both cellular and clonal shape homeostasis in an identical manner. Moreover, we numerically found that the interface homeostasis are counterbalanced by the differential cell contractility: increased cell contractility of faster dividing cells suppressed expansion of their area, leading to the decrease of interface smoothness and the cell anisotropy.

330B

Pumilio regulates regenerative growth in *Drosophila* imaginal discs. Syeda Nayab Fatima Abidi, Amanda Brock, Rachel Smith-Bolton. Cell and Developmental Biology Dept, University of Illinois at Urbana-Champaign, Urbana, IL.

Drosophila melanogaster imaginal discs provide a useful model for studying tissue regeneration. The process of regeneration in *Drosophila* has largely been studied by fragmenting imaginal discs, culturing them *in vivo* in the abdomen of adult females and finally transplanting them into third instar larvae to allow differentiation. However, this experimental method is impractical for performing large-scale genetic screens. We have developed a novel and efficient method to induce tissue damage genetically in the *Drosophila* larval wing imaginal disc under precise spatial and temporal control. Using our ablation system we performed an unbiased genetic screen of isogenic deficiencies of the third chromosome for dominant modifiers of imaginal disc regeneration. Our screen has identified 19 mutants that do not allow the wings to regenerate fully, including Df(3R)ED5331. We have mapped the poor-regeneration phenotype observed in *Df(3R)ED5331/+* animals to the *pumilio* gene locus. Pumilio is an RNA-binding protein that primarily acts as a translational repressor by binding the 3' UTRs of multiple mRNAs, and can regulate cell growth and cell division in other contexts. We are characterizing how reduction in *pumilio*levels results in impaired regeneration. In damaged imaginal wing discs, the regeneration blastema, or site of active proliferation, forms at the wound site, while proliferation is inhibited outside of the blastema. Interestingly, in *pumilio/+* mutants this inhibition of proliferation at a distance from the wound was reduced. Current work is aimed at understanding how this change in proliferation outside the blastema is regulated by *pumilio*, and whether it contributes to the impairment of regeneration in *pumilio/+* discs. In addition to studying regeneration in the wing imaginal disc, we have also developed an ablation system in the antennal imaginal disc to determine whether modifiers of regeneration identified in the wing disc, such as *pumilio*, are important in multiple tissues. Our work will provide insight into the factors that regulate epithelial tissue regeneration.

331C

A genetic screen in wing imaginal discs for regeneration genes. Amanda R. Brock, Mabel Seto, Rachel K. Smith-Bolton. Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

Regeneration is a complex process that requires an organism not only to recognize and repair tissue damage, but also to grow and pattern new tissue. Here we describe a system of genetically induced ablation in the *Drosophila* larval imaginal wing disc that enables us to perform large-scale genetic screens to identify novel regulators of regeneration. We ablate the wing primordium by inducing apoptosis in a spatially and temporally controlled manner, after which we stop apoptosis, allow regeneration to occur, and assess the amount and quality of regeneration by examining the adult wings. In order to identify genes that regulate regeneration, we have carried out a dominant modifier screen using isogenic deficiencies covering the right arm of the third chromosome. We have completed a screen of the right arm of the third chromosome. To date we have identified 21 regions that contain a gene that modifies the regenerative response. Here we present the results of our screen, plus the initial characterization of *cap-n-collar*, a transcription factor that is required for regenerative growth in both flies and mammals. We will use the genetic power of *Drosophila* to elucidate the mechanism by which this gene regulates regeneration. .

332A

Investigating the role of Extracellular Matrix (ECM) remodeling during wing disc regeneration in *Drosophila*. Amanda Sul, Sumbul Khan, Rachel Smith-Bolton. University of Illinois at Urbana-Champaign, Urbana, IL.

Some animals are capable of regenerating parts of their body after damage, whereas other animals are only capable of wound healing and scarring. Tissue regeneration is the replacement of damaged tissue and restoration of morphology as well as functionality. Regeneration requires a complex interplay between extracellular matrix (ECM) and cells within a tissue. Matrix metalloproteinases (MMPs) are responsible for degrading the ECM and are highly expressed at wound sites. However, the exact role of the ECM and MMPs in the wound healing process is not yet clear. We employ a non-surgical method of inducing tissue damage and regeneration in the wing imaginal disc of *Drosophila*, which is highly amenable to genetic manipulations. Using this system we tracked the expression of Viking (Vkg), the gene coding for ECM component Collage IV, during different stages of regeneration after tissue damage. We observed disruptions in the basement membrane structure as assayed by the Green Fluorescent Protein (GFP) tagged reporter Vkg-GFP. We also observed changes in matrix metalloproteinase 1 (Mmp1) protein expression levels during various stages of regeneration. We are further studying the effects of overexpressing or inhibiting Mmp1 expression to understand the functional significance of Mmp1 in the regenerating wing discs. Finally we have examined the role of Jun N terminal Kinase (JNK) signaling in ECM remodeling during regeneration.

333B

Abscission is regulated by the ESCRT-III protein Shrub in Drosophila germline stem cells. Neuza Reis Matias[1,2], Juliette Mathieu[1,2], Jean-René Huynh[1,2]. 1) Department of Genetics and Developmental Biology, Institut Curie, Paris, France; 2) CNRS UMR3215; Inserm U934 F-75248 Paris, France.

Abscission is the final event of cytokinesis that leads to the physical separation of the two daughter cells. Recent technical advances have allowed a better understanding of the cellular and molecular events leading to abscission in isolated yeast or mammalian cells. However, how abscission is regulated in different cell types or in a developing organism remains poorly understood. Here, we characterized the function of the ESCRT-III protein Shrub during cytokinesis in germ cells undergoing a series of complete and incomplete divisions. We found that Shrub is required for complete abscission, and that levels of Shrub are critical for proper timing of

abscission. Loss or gain of Shrub delays abscission in germline stem cells (GSCs), and leads to the formation of stemcysts, where daughter cells share the same cytoplasm as the mother stem cell and cannot differentiate. In addition, our results indicate a negative regulation of Shrub by the Aurora B kinase during GSC abscission. Finally, we found that Lethal giant discs (lgd), known to be required for Shrub function in the endosomal pathway, also regulates the duration of abscission in GSCs.

334C

Deciphering the Functional Collaboration of Mid and Bric-a-Brac 2 as Potential Regulators of Cellular Proliferation within Adult *Drosophila* Ovaries. Petra Visic, Sandra Leal. Biological Sciences, University of Southern Mississippi, Hattiesburg, MS.

Stem cell niches are highly organized and specialized stem cell microenvironments detected within specific tissues [1]. In *Drosophila melanogaster*, three distinct stem cell niches are located within the ovary including the germline stem cell (GSC), follicle stem cell (FSC), and escort stem cell (ESC) niches. Recently, Lomas et al. [2] reported that gurken/epidermal growth factor receptor (EGFR) signaling is modulated within posterior ovarian follicle cells by Midline (Mid). The Mid transcription factor had not previously been associated with the EGFR pathway.The *mid* gene encodes a T-box transcription factor protein that specifies cell fates in the developing heart [10,11], central nervous system [12,13], epidermis [14] and eye of *Drosophila* [15].The *Tbx20* gene represents the conserved vertebrate ortholog of *mid*. Experimental evidence suggests that Tbx20 regulates cell proliferation (CP) within the embryonic chamber myocardium of the mouse; Tbx20 null mice exhibit increased expression of Tbx2 and in turn, decreased levels of the proto-oncogene N-myc-1 leading to hypoplasia [16]. We undertook a genetic modifier screen and discovered that *mid* interacts with several genes implicated in the control of CP including *extramacrochaetae* [17,18,19] and *dFOXO* [20]. In addition, the modENCODE consortium identified *bric-a-brac 1* and *2* (*bab-1* and *bab-2*) as theoretical *mid*-interacting genes that encode proteins harboring a BTB/POZ-ZF domain in *Drosophila* – a domain associated with oncogenic activity in humans [21,22]. We carried out *mid* and *bab2* loss-of-function and gain-of-function studies specifically within ovaries using the UAS-Gal4-binary expression system [23]. Wild-type and mutant ovaries were dissected and subjected to a 5- Bromo-2'- deoxyuridine (BRdU) proliferation assay. Our results show that misexpressing *bab*-2 within the ovarian GSC niche is coupled with increased Mid expression and decreased levels of CP. We also co-labeled BRdU treated wild-type and mutant tissues with anti-c-Myc antibody.Misexpressing *mid* results in increased levels of c-Myc. Thus, we conclude that Mid functions within pathways regulating CP of the GSC niche.

335A

Intertissue control of the nucleolus via a myokine-dependent longevity pathway. Fabio Demontis[1], Vishal K. Patel[2], William R. Swindell[3], Norbert Perrimon[2]. 1) Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA; 2) Department of Genetics, Harvard Medical School/HHMI, Boston, MA 02115, USA; 3) Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI 48109, USA.

Recent evidence indicates that skeletal muscle influences systemic aging, but little is known about the signaling pathways and muscle-released cytokines (myokines) responsible for this intertissue communication. Here, we show that muscle-specific overexpression of the transcription factor Mnt decreases age-related climbing defects and extends lifespan in Drosophila. Mnt overexpression in muscle autonomously decreases the expression of nucleolar components and systemically decreases rRNA levels and the size of the nucleolus in adipocytes. This nonautonomous control of the nucleolus, a regulator of ribosome biogenesis and lifespan, relies on Myoglianin, a myokine induced by Mnt and orthologous to human GDF11 and Myostatin. Myoglianin overexpression in muscle extends lifespan and decreases nucleolar size in adipocytes by activating p38 mitogen-activated protein kinase (MAPK), whereas Myoglianin RNAi in muscle has converse effects. Altogether, these findings highlight a key role for myokine signaling in the integration of signaling events in muscle and distant tissues during aging.

336B

Heart function, flight performance, and stress resistance in *Drosophila*. James N Kezos, Larry G Cabral, Laurence D Mueller, Michael R Rose. Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA.

Examining the physiological interrelationships of cardiac function, athletic performance, and stress resistance in *Drosophila* should be of value for understanding cardiac diseases and their prevention. But before we can focus on cardiac disease and prevention, more information on the mechanisms of cardiac function and their relationship to other physiological processes is needed. By conducting electrical pacing and flight exhaustion assays with manipulative conditioning, we have started to unpack the physiological interrelationships between heart function, athletic performance, and such functional characters as longevity and stress resistance. Flight to exhaustion, desiccation, and starvation are three kinds of stress that we applied prior to electrical cardiac pacing assays. Desiccation and starvation were also applied prior to flight exhaustion assays. In addition, a cohort of flies from each population was flown to exhaustion and then placed in either the desiccation or starvation stress environment. It was determined that flight exhaustion and exposure to desiccation do not affect the rate of cardiac arrest. This implies that glycogen is not an important determinant of cardiac robustness, because it is used up in the course of flight and desiccation. Starved fruit flies had reduced rates of cardiac arrest. This implicates lipids as a factor impinging on heart function, because starvation resistance is chiefly determined by lipid reserves. Coconut oil was fed to adult *Drosophila* populations for four days, followed by electrical cardiac pacing. We found that exposure to a high-fat diet significantly increased cardiac arrest rates, as have others. This further corroborates the hypothesis that fat reserves impinge on heart function in *Drosophila*. Performing these manipulative experiments has allowed us to infer which particular stressors reduce or improve heart function, as well as the role of metabolic reserves in cardiac function. .

337C

***Drosophila* p38 MAP Kinase Regulates Age-dependent Protein Homeostasis. Sarah M. Ryan**[1], Amelia M. Burch[2], Subhabrata Sanyal[2,3], Alysia D. Vrailas-Mortimer[1,2]. 1) Department of Biological Sciences, University of Denver, Denver, CO; 2) Department of Cell Biology, Emory University, Atlanta, GA; 3) BiogenIdec, Boston, MA.

Maintaining a properly balanced proteome is an important aspect of cellular health and function. Disruptions in protein homeostasis, such as aging or exposure to oxidative stress, can lead to the accumulation of damaged or misfolded proteins, which in turn can form protein aggregates. These aggregates are thought to be toxic and potentially lead to an aged or diseased state, such as Alzheimer's disease, Parkinson's disease and ALS. Therefore it is critical for these damaged proteins to be properly degraded or cleared from the cell. One protein quality control mechanism is the Chaperone Assisted Selective Autophagy (CASA) complex which targets damaged proteins for destruction via the autophagosome/lysosome. The *Drosophila* CASA complex consists of the chaperones HspB8 and Hsc70 and the nucleotide exchange factor, Starvin. We find that HspB8 physically interacts with the p38 MAP Kinase (p38K), which we have previously shown to regulate aging and oxidative stress. In addition, we find that p38K colocalizes with CASA complex members at the Z-disk of the adult flight muscle and are testing these interactions in the adult brain. We also find that p38K regulates protein homeostasis in response to natural aging and oxidative stress. Finally, we find that p38K genetically interacts with the CASA complex to regulate lifespan and protein homeostasis. As p38K has been implicated in many age-dependent neurodegenerative diseases, our results suggest that altered p38K function may contribute to the accumulation of protein aggregates, potentially leading to a disease state.

338A

Fecundity vs. Starvation Resistance: Resource Allocation in Starvation-Selected *Drosophila*. Timothy J. Saitta, Allen G. Gibbs. Univesity of Nevada, Las Vegas School of Life Sciences 4505 Maryland Parkway Las Vegas, NV 89154.

Selection for starvation resistance in *Drosophila melanogaster* has resulted in greater acquisition of resources in the form of lipids. We questioned how these resources are allocated into maintenance and storage or gamete production. Preliminary observations led to our hypothesis that starvation selection shifts the energy balance away from reproduction and towards starvation survival. We tested 3 starvation selected and 3 fed control replicate populations for lipid content, development time, fecundity and starvation survival. Starvation selected flies took an average of 23 hours longer gathering resources in their larval stage, and were consequently fatter after eclosion. Starvation-selected flies also laid fewer eggs than controls. Within each population, female flies who laid fewer eggs survived starvation longer than high-fecundity females. This indicates that energetic resources in starvation-selected flies are not allocated equally, but rather toward survival in a trade-off with reproduction. Supported by NSF awards 1358896, and IOS-1355210.

339B

Innate immune aging mediated by ecdysone signaling in response to water stress. Wenjing Zheng[2], Florentina Rus[1], Marc Tatar[2], **Neal Silverman**[1]. 1) Med/Div Infectious Dis, Univ Massachusetts Med Sch, Worcester, MA; 2) Dept. Ecology and Evol. Biology Brown University.

Old *Drosophila* display elevated levels of antimicrobial peptide gene expression. In fact, Malpighian tubules (MT) dissected from old adults are more immune responsive when challenged with Imd agonists. The immune sensitivity of MT is modulated by ecdysone signaling in response to water stress in young adults, while the MT of aged adults intrinsically exhibit high immune sensitivity. Withholding water from young adults for 2 hours (water stress) is sufficient to significantly increase MT expression of the innate immune receptor PGRP-LC, an established target of the ecdysone signaling cascade. Based on RNAi analyses, ecdysone receptor expression is required in the MT for the induction PGRP-LC in response to water stress. Adult flies (males and females) increase ecdysone production when water stressed, apparently from several somatic tissues including the MT, and this somatic production of ecdysone is sufficient to induce PGRP-LC transcription. Notably, ecdysone is elevated in aged flies, and an old literature suggests that aged flies are intrinsically water stressed. Accordingly, we aged cohorts of flies under three levels of relative humidity and measured expression of PGRP-LC in the MT. PGRP-LC mRNA increased with age in all groups, and was likewise reduced overall by higher humidity. Critically, high humidity significantly blunted the effect of aging upon PGRP-LC expression in the MT. Together these results suggest that elevated innate immune responses with age are caused in part by elevated PGRP-LC levels induced by ecdysone as a response to water stress.

340C

Arginine methyltransferases regulate longevity and stress resistance in *Drosophila melanogaster*. Su Yeun Yu[1], Gwang-Ic Son[1], Bodokhsuren Tsogbadrakh[2], Yunjeong Kim[1], Joong-Jean Park[3], Yongchul Lim[4], Eunil Lee[1]. 1) Preventive medicine, College of Medicine, Korea University, Seoul, Korea; 2) Department of Internal medicine, Seoul National University Hospital, Seoul, Korea; 3) Department of Physiology, College of Medicine, Korea University, Seoul, Korea; 4) Department of Surgery, Samsung Medical Center, Sungkyunkwan University, School of Medicine, Seoul, Korea.

Protein arginine methyltransferase (PRMT) catalyze the methylation of arginine residues to asymmetric dimethyl-arginines, especially, PRMT1 and 4 can methylate histone and steroid hormone receptor as a coactivator. We previously reported that the expression and activity of PRMT1 and 4 were significantly reduced in replicatively senescent fibroblasts and tissues from 24-month-old rats, compared to the young. However, it is unclear whether the altered expression of age-dependent manner is related with aging and metabolism. To approach this underlying mechanisms, we investigated the effects of*Drosophila* arginine methyltransferase 1 and 4 (Art1 and 4) on the stress tolerance and lifespan in *Drosophila melanogaster* model system. The each sequence and substrate specificity of *Art1* and *4* is highly similar to mammalian *PRMT1* and *4*. Using GAL4/UAS system, we found that the conditional inhibition of *Art1* and *4* in adult flies

by using RNA interference lines decreased lifespan and resistance to starvation and oxidative stress. Furthermore, we generated transgenic flies over-expressing specifically *Art1* and *4* for analyzing their sensitivity to stresses and lifespan, comparing to knocking down results. The ubiquitous over-expression of *Art1* and *4* significantly increased stress tolerance. Importantly, these phenotypic effects by genetic modulation of *Art1* and *4* were observed in sex-dependant manner. Significantly, the stress tolerance and lifespan in *Drosophila* were governed by *Art1* mutant males and *Art4* mutant females. We conclude that *Art1* and *4* play a role to determine stress tolerance and lifespan and their activity is possibly limited under sex specificity in *Drosophila melanogaster*.

342B

The biological roles of Iron-Sulfer protein in *Drosophila*. Kai-Ting Huang[1,2], Jian-Chiuan Li[1], Hsiao-Yen Chan[1], Chu-Ya Cheng[1], Po-Lin Chen[1], Ya-Chen Lin[1,2], Horng-Dar Wang[2], Chun-Hong Chen[1]. 1) National Health Research Institutes, Miaoli, Taiwan; 2) National Tsing Hua University, Hsinchu, Taiwan.

There are three iron-sulfer proteins, CISD1, CISD2 and CISD3 in mammalian (Lin et al, 2007). The members of this family all contain the CDGSH iron sulfur domain which consists of iron-sulfur (Fe-S) clusters for binding these two metals onto the protein. In mitoNEET, the CDGSH iron sulfur domain 1 protein is an integral membrane protein located on the outer mitochondrial membrane whose function may be to transport iron into the mitochondria. In *Drosophila*, there are two proteins, in this protein family, called CG1458(dcisd2) and CG3420(dcisd3). The CG1458(dcisd2) contains two MitoNEET domain in the N terminal region and one CDGSH domain in C terminal region. Ortholog of CG1458(dcisd2) in mammalian is more similar to mouse Cisd2 than mCisd1 with the MitoNEET domain. Another CDGSH protein, CG3420 contains two CGDSH domain, it shares more similarity with Cisd3, we named it as dcisd3. Here, we characterized the subcelluar localization of these proteins in the flight muscle. Overexpression of these CDGSH proteins might result mitochondria dynamic change. How these proteins involved in mitochondrial metabolism and dynamic change will be discussed in this report. .

343C

Signaling and starvation: effects of activity reduction of central metabolic pathway genes in AKH and dILP2 producing cells on starvation resistance. Erik Lavington[1], Eugene Brud[2], Matthew Talbert[3], Walter Eanes[2]. 1) Graduate Program in Genetics, Stony Brook University, Stony Brook, NY; 2) Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY; 3) Department of Biology, University of Louisiana at Monroe, Monroe, LA.

Starvation in *Drosophila melanogaster* has been used to identify tissues and genes involved in organismal level nutrient sensing. Ablation of adipokinetic hormone (AKH) or Drosophila Insulin-like protein (dILP) producing cells results in increased starvation resistance. In starvation resistance studies, altered metabolism is observed as an effect of altered starvation resistance. How altered metabolism can affect, if at all, starvation resistance is unclear. We used P-element excision mediated mutagenesis and RNAi to reduce enzyme activity in whole-body and specific tissues, respectively. Tissue specific knock down was performed using Akh-GAL4 for corpora cardiaca (CC) specificity and dilp2-GAL4 for median neurosecretory cell (mNSC) specificity. We found significant differences between low- and normal enzyme activity p-element lines in starvation resistance for all six of the central metabolic genes we tested. However, lower enzyme genotype activity does not generally lead to increased, or decreased, starvation resistance. We further show that RNAi knockdown of central metabolic genes in CC cells and mNSCs yields different results. Generally, RNAi knockdown of this set of genes in mNSCs does not produce a significant difference from controls. RNAi knockdown of the same genes in the CC results in a significant difference in starvation resistance for some, but not all, of these genes. These results demonstrate the capacity of central metabolic enzyme activity to modulate starvation resistance.

344A

The *Drosophila Lactate Dehydrogenase* gene promotes production of the oncometabolite 2-hydroxyglutarate during normal juvenile growth. Hongde Li, Alexander Hurlburt, Jason Tennessen. Indiana University Bloomington, IN 47405.

The metabolism of proliferating cancer cells not only generates energy, but also synthesizes the biomolecules required for growth. In response to these metabolic demands, cancer cells rely on aerobic glycolysis, a metabolic program that synthesizes biomolecules from carbohydrates. *Drosophila* also uses aerobic glycolysis to promote the nearly 200-fold increase in biomass that occurs during larval development, and it has emerged as an ideal model to study this conserved metabolic program. We are using larval development as a model to understand how the inhibition of aerobic glycolysis affects rapid growth and animal physiology. Our initial studies are focused on inhibiting key enzymes involved in aerobic glycolysis, many of which are also potential therapeutic targets. Toward this goal, we have determined that mutations in *Lactate Dehydrogenase* (*Ldh*; also known as *ImpL3*) significantly disrupt aerobic glycolysis and induce an L3 lethal phase. Intriguingly, our GC-MS-based metabolomic analysis of the *Ldh* mutants revealed an unexpected link between the production of lactate and the oncometabolite 2-hydroxyglutarate (2-HG). This compound acts as a competitive inhibitor of 2-oxoglutarate-dependent dioxygenases, suggesting that LDH-dependent 2-HG production could link glycolytic flux with genome-wide changes in histone methylation and gene expression. Intriguingly, although 2-HG synthesis occurs in cancer cells that harbor neomorphic IDH1 mutations, it has never been linked with normal developmental growth. Our findings suggest that rather than an aberrant metabolite produced in cancer cells, 2-HG is part of a conserved metabolic program that contributes to organismal growth and aerobic glycolysis.

345B

Regulation of lipid and energy homeostasis by the nuclear receptor DHR78. Stefanie Marxreiter, Carl S. Thummel. Human Genetics, University of Utah, Salt Lake City, UT.

DHR78 encodes the single fly ortholog of the mammalian orphan nuclear receptors TR2 and TR4. Recent studies of TR4 suggest that it regulates a metabolic transcriptional program. *TR4* mutant mice display protection against obesity when placed on a high fat diet and disruption of mitochondrial function. However, the mechanisms by which this is occurring have not yet been elucidated. Consistent with a potential role in metabolism, antibody staining reveals that DHR78 is expressed in the fat body, Malpighian tubules, muscle, and throughout the intestine. In addition, similar to the phenotypes observed in*TR4* mutant mice, *DHR78* mutants fail to increase their lipid stores under obesogenic conditions. Adult mutant females are sensitive to starvation, have reduced triglycerides, and display reduced fertility, with an apparent block in oogenesis near the onset of vitellogenesis. These phenotypes implicate a role for DHR78 in the regulation of lipid metabolism, potentially in lipid transport. Consistent with this hypothesis, northern blot analysis reveals reduced expression of key lipid transport genes such as *lipophorin* (LPP) and *microsomal tricylglyceride transfer protein* (MTP). Finally, decreased motility and a failure to fly in *DHR78* mutants implicates a role in proper mitochondrial function, providing another parallel with *TR4* mutant mice. Taken together, our results suggest an evolutionarily-conserved function for DHR78 in energy homeostasis. Current efforts are focused on defining the molecular mechanisms by which DHR78 regulates lipid metabolism and mitochondrial function.

346C

Metabolic studies and the insulin pathway in *Drosophila* species. María T. Peña-Rangel, Deyannira Otero-Moreno, Juan Manuel Murillo-Maldonado, Juan R. Riesgo-Escovar. Neurobiologia del Desarrollo y Neurofisiología, Universidad Nacional Autónoma de México, Querétaro, Querétaro, Mexico.

Metabolism regulation is essential for life. The basic metabolic processes are highly conserved in eukarya, yet much remains to be understood. Metabolic dysfunction associated with metabolic syndrome, insulin resistance, obesity, and diabetes is now common in many human populations. Just as in humans, *Drosophila melanogaster* regulates carbohydrate and lipid levels. It has analogous organs to vertebrate liver, adipose tissue, kidneys, gut, and pancreas. We used insulin pathway viable hypomorphic heteroallelic *D. melanogaster* combinations to study the onset and development of metabolic disorders after embryogenesis throughout the life cycle. We measured carbohydrates and lipid levels in embryo, second and third instar larvae, pupae and adults of several ages. We evaluated different diets in mutant flies, measuring lifespan, carbohydrate and lipid levels. For this, we cultured first instar larvae of the different genotypes in eight different isocaloric diets containing different amounts of proteins and lipids. Survival curves were built starting from these first instar larvae cultured throughout the life cycle in the same diets. Our results show that metabolic dysfunction begins during larval stages, and diet composition does have an effect on survival. As a comparison, we also studied the same metabolic parameters in another Drosophila species, *Drosophila lutzii*, which is not saprophytic, but rather, feeds on flowers. Our results show that these flies accumulate abnormal amounts of carbohydrates when cultured in regular *D. melanogaster* food. The study was supported by PAPIIT # IN200313-25 and UNAM funds.

347A

***dFatp* Regulates Nutrient Distribution and Long-term Physiology in *Drosophila*. Chelsea R. Richardson**, Alyson L. Sujkowski, Robert J. Wessells. Physiology, Wayne State University, Detroit, MI.

Onset and progression of age-related functional declines are closely regulated by nutrient allocation and usage. Reduced expression in *Drosophila fatty-acid transporter protein* (*dFatp*) alters nutrient distribution and lipid metabolism. Heterozygous *dFatp* mutants ($dFatp^{mut}$) display improvements in multiple aspects of physiology, including lifespan, stress resistance, and mobility. Such flies also have altered lipid metabolism with increased whole-fly triglycerides and accumulation of lipid droplets in cardiac muscle despite a reduced feeding rate. Lipid accumulation in the heart leads to reduced fractional shortening and cardiac stress tolerance in $dFatp^{mut}$ flies. Endurance training reduces cardiac lipids levels and reverses the cardiac impairments without altering the extended lifespan. Using lipidomics, overexpression, and structure/function analysis, we are currently working to better understand the mechanism of lifespan extension in these mutants.

348B

Age and Gender Effects on Anoxia Tolerance in *Drosophila melanogaster* Mirror Patterns in Mammals. James C. Sargent, Jacob B. Campbell, Jon F. Harrison. School of Life Sciences, Arizona State University, Tempe, AZ 85287.

Cell death occurring from anoxia is considered to be the cause of many human diseases such as heart attack and stroke. Though much is known about the cause of anoxia in such conditions, we still have a poor understanding of the mechanisms causing cell death and the genetic and physiological processes responsible for variation in survival of anoxia. In rodents, gender has a strong effect on anoxia tolerance, apparently due to the neuroprotective and neurotropic effects of estrogen signaling. Age also has powerful effects on anoxia survival in rodents, with younger mammals generally more tolerant of anoxia, with this effect attributed variously to differences in circulation, metabolism and chaperone induction. *Drosophila melanogaster* are particularly interesting models for studying responses to anoxia as they can survive many hours of anoxia and most of their metabolic pathways are identical to those of humans. We hypothesized that females would have a higher tolerance to anoxia than males even though estrogen-signaling is not gender-specific in *Drosophila*. We also hypothesized, based on work with mammals, that younger adults would have a higher anoxia tolerance than older adults. We exposed adult*Drosophila*, ages 1, 3, 5, 7, 9, and 12 days old, to six hours of anoxia. Survival was assessed 24 hours post-

treatment; surviving individuals exhibited movement if stimulated. As predicted, 50% of females survived anoxia, while only 25% of males survived (pooling across all ages). Survival declined with age in a linear fashion for females and in a more exponential fashion for males. Seventy nine percent of adults one day past eclosion survived six hours of anoxia; while only 10% of twelve-day-old adults survived. These data show that patterns of gender- and age-associated variation in tolerance to anoxia are similar in *Drosophila* and mammals, suggesting that *Drosophila* may be underutilized models for studies of the genetic and biochemical mechanisms of pathology of stroke and heart disease. This research was partially supported by NSF 1256745 to JFH.

349C

***Drosophila* metabolic and transcriptomic responses to its gut symbiont *Lactobacillus plantarum* point enhancement of host dietary protein digestion and fatty acids beta-oxidation as key features of bacterial growth-promoting effect. Gilles Storelli**, François Leulier. IGFL/ENS Lyon, Lyon Cedex 07, Lyon, France.

It is widely recognized that intestinal microbiota influences its host biology, with impacts on metabolic, tissular and immune homeostasis. However, the characterization of prokaryotic and eukaryotic factors involved in this mutualistic relationship, and more precisely those influencing host fitness, is hampered by the great complexity found in mammalian intestinal ecosystems. In this regard, we use simple association models that are axenic *Drosophila* embryos associated with single strains of *Lactobacillus plantarum*, a major representative of *Drosophila* natural microbiota. We previously showed that*L.plantarum* sustains larval systemic growth rate and maturation despite nutrient scarcity by modulating Ecdysone and dILPs production through host TOR-dependent nutrient sensing. Moreover, growth-promotion is strain-specific: testing a collection of *L.plantarum* isolates revealed a wide range in their propensity for growth sustainment, illustrating variations in the amplitude of host response to different strains of the same commensal species. To dissect the host response to different *L.plantarum* strains, we associated embryos with two reference isolates, one particularly potent for growth promotion and another showing a markedly reduced effect on juvenile growth, and performed RNAseq and metabolites profiling at various time points during larval development. Our results suggest a common trend in the host responses to the two strains, but with differences in their magnitude: mono-associated animals show clear signatures of altered oxidative metabolism, notably fatty acid beta-oxidation, concomitant with a shift in the systemic redox balance and antioxidant defenses. Moreover, our results reveal a marked activation of intestinal proteolytic activity upon association. Taken together our results support the idea that*L.plantarum* association optimizes host dietary protein digestion and lipid catabolism to support anabolic growth during larval stages. We are currently dissecting how these nutritional and metabolic features translate into enhanced TOR signaling and accelerated host maturation.

350A

Drosophila Mitochondrial Pyruvate Carrier mutants display defects in carbohydrate metabolism and hallmarks of diabetes. Dona Wisidagama, Daniel K. Bricker, Carl S. Thummel. Human Genetics, University of Utah, Salt Lake city, UT.

Pyruvate, the end product of glycolysis, is a substrate for catabolic and anabolic pathways. Disruption of pyruvate entry into the mitochondria will result in reduced TCA cycle activity and a decreased cellular ATP/ADP ratio. Reduced pyruvate oxidation is also associated with decreased glucose stimulated insulin secretion and peripheral insulin resistance, metabolic disorders that can lead to obesity and diabetes. Recently our lab discovered the Mitochondrial Pyruvate Carrier (MPC) that is responsible for transporting cytosolic pyruvate into the matrix of the mitochondria, linking glycolysis with the TCA cycle. *Drosophila MPC1* deletion mutants are viable on standard fly food but show rapid lethality on a high sugar diet. Mutant animals have low ATP, triacylglycerol (TAG), and protein compared to wild-type animals, but high trehalose, glucose and glycogen levels, indicating a defect in carbohydrate metabolism. These mutants also display multiple hallmarks of diabetes, including hyperglycemia, increased sorbitol and glucose intolerance. They also show reduced peripheral insulin signaling as revealed by decreased phosphorylated AKT. Currently we are investigating whether the hyperglycemia observed in*dMPC1* mutants is a result of reduced pyruvate oxidation and/or peripheral tissue insulin resistance. These studies will provide insights into the role of pyruvate metabolism in carbohydrate homeostasis in*Drosophila* as well as new directions for understanding the molecular basis of diabetes.

351B

Hsc70 regulates fat metabolism during *Drosophila* aging. Yan Yan[1], Hao Wang[2], Renjie Jiao[1,3]. 1) State Key Laboratory of Brain and Cognitive Scienc, Institute of Biophysics, the Chinese Academy of Sc, Beijing, China; 2) Department of Chemistry and Biology, National University of Defense Technology, Changsha 410072, China; 3) Guangzhou Hoffmann Institute of Immunology, School of Basic Sciences, Guangzhou Medical University, Dongfengxi Road 195, Guangzhou 510182, China.

The progressive ectopic fat accumulation (EFA) contributes to age-related physiological dysfunction and exacerbates the progression of human diseases such as obesity, diabetes and cancer. It is known that impaired function of adipose tissue can cause disease-related EFA, however, the primary cue(s) of age-related EFA remains largely elusive. To understand how age-related EFA occurs at the molecular level, we first characterized age-related EFA in *Drosophila*, and identified a handful of genes involved in this process through a candidate screen. Our results show that loss of autophagy-related genes (Atg1 and Atg5) or the chaperone protein, Hsc70, led to significant fat accumulation during aging. Hsc70 maintained the protein level of lipid droplet (LD) resident proteins during aging. Moreover, overexpression of Hsc70 ameliorated EFA-induced physiological dysfunction such as inflammation and stress susceptibility during aging. These findings suggest that aberrant protein quality control may serve as an intrinsic cue to cause age-related EFA, and intervention of the protein quality can be a promising approach to treat age-related metabolic diseases such as obesity and diabetes.

352C

Absence of a peritrophic matrix in *drop-dead* mutant flies. Sean Conway[1], Christine L. Sansone[1], Johan Billen[2], Jozef Vanden Broeck[2], Edward M. Blumenthal[1,2]. 1) Biological Sciences, Marquette University, Milwaukee, WI; 2) Zoological Institute, University of Leuven, Leuven, Belgium.

Drosophila carrying a mutation in *drop-dead* (*drd*) exhibit phenotypes that include adult lethality, female sterility, neurodegeneration, and defective gut function. Defective gut function is characterized by a lower rate of defecation and accumulation of ingested food in the crop. To further investigate this phenotype, we examined the peritrophic matrix (PM), a chitin- and protein-containing barrier structure that lines the inside of the midgut and is believed to protect the gut and to aid in digestion. Control and *drd* mutant flies were examined by three techniques: gross dissection of the midgut, histology of semi-thin sections, and staining of dissected midguts with the chitin-binding dye calcofluor. By all of these measures, the PM was absent from flies carrying severe alleles of *drd*. Histology of the anterior midgut also revealed gross distension of the cardia and the presence of diffuse material that could represent uncondensed PM components. To determine the location of *drd* expression required for PM formation and normal gut function, RNAi of *drd* was driven by gal4 lines that express in the anterior gut. One driver, *DJ626-gal4*, defined a pattern of *drd* expression that was both necessary and sufficient for normal gut function. Knockdown of *drd* with *DJ626-gal4* resulted in short-lived flies with reduced defecation rates and no PM. Conversely, rescue of *drd* expression with *DJ626-gal4* resulted in rescue of both defecation rate and PM presence. Visualization of the *DJ626-gal4* expression pattern indicated strong expression in the most anterior midgut cells of the cardia, a subregion previously associated with PM synthesis. In contrast, drivers that failed to rescue gut function were not expressed in the anterior cardia. Thus expression of *drd* in the anterior cardia is required for PM synthesis and normal passage of food through the gut. Furthermore, the absence of the PM in *drd* mutants is a phenotype unreported for any other *Drosophila*gene. *Supported by NSF IOS-1355087 and a senior fellowship (SF/11/008) of the KU Leuven Research Fund to EMB.*.

353A

Stunted is a fat body-derived factor that remotely couples insulin secretion with nutrient availability through its receptor Methuselah. Renald Delanoue, Neha Agrawal, Meschi Eleonore, Leopold Pierre. iBV, CNRS UMR 7277/INSERM UMR 1091/UNS, Nice, France.

Body growth is tightly regulated by nutrient availability. Upon nutritional shortage, animals harmoniously reduce their body size, suggesting that global mechanisms involving hormonal control are at play. The insulin/IGF molecules set animal's growth rate and coordinate body growth to nutrition. Drosophila has a conserved insulin/IGF system with 8 insulin-like peptides called Dilps, a unique insulin receptor (dInR) and a conserved downstream signalling cascade. Several *dilp* genes are produced by clusters of neurosecretory cells in the fly brain called the Insulin-Producing Cells (IPCs). Amino acids are limiting for larval growth. Under rich food, Dilps are released from the IPCs into the hemolymph and promote growth, a process that is blocked upon shortage for amino acids. Changes in dietary amino acid levels are relayed by an organ called the fat body (FB), which couples nutrition to Dilp release through the emission of FB-derived factors (FBFs). In order to identify the molecular nature of these signals, we carried out RNAi screens both in the fat body and in the brain IPCs. We identified Methuselah (Mth) as a G protein-coupled receptor required in the brain IPCs for proper nutrient coupling: in absence of Mth function, the brain IPCs fail to promote Dilp secretion in response to high amino acid diet. Interestingly, expression of the Mth ligand Stunted (Sun) in the FB is required for normal brain Dilp secretion. The Sun peptide is detected in the hemolymph of well fed larvae, but absent when animals are fed low amino acid diet. Experiments using dissected brains co-cultured with larval hemolymph reveal that Sun is both necessary and sufficient to promote Dilp release from brain IPCs. Intriguingly, the Sun peptide has a separate function as the epsilon subunit of the mitochondrial F_1F_0-ATP synthase and is readily detected in the mitochondria of fat cells. This raises the possibility that Sun could link mitochondrial energy status in the fat body with the control of circulating insulin levels via its receptor Mth in the brain IPCs.

354B

FLIC: High-throughput, continuous analysis of Drosophila feeding behaviors. Jennifer Ro[1], Zachary Harvanek[3], **Scott Pletcher**[2]. 1) Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, Michigan; 2) Department of Molecular and Integrative Physiology and Geriatrics Center, University of Michigan, Ann Arbor, Michigan; 3) Medical Scientist Training Program, University of Michigan, Ann Arbor, Michigan.

Feeding behavior integrates complex processes that include physiological demand, sensory perception, food choice, and motor control. Indeed, the rich biological insights that may be drawn from a detailed description of an organisms' feeding activity have motivated scientists to quantify certain aspects of this behavior as meaningful outputs in fields as diverse as neurobiology, metabolism, and aging. Most existing methods for measuring feeding behaviors are, however, limited in their ability to provide a detailed picture of an individual fly's behavioral interactions with its nutritional environment in undisturbed, steady state conditions. They are also quite labor intensive and difficult to "scale-up" and automate for genetic or pharmacological screens. To address these limitations we have developed the FLIC (Fly Liquid-Food Interaction Counter), a general purpose system for accurately and continuously measuring feeding behaviors. The FLIC device uses a simple electronic circuit that is monitored continuously (every 5us) to signal when a single fly interacts with a liquid food, and the system has the capacity for simultaneous, automated analysis of thousands of flies. We thus obtain continuous trajectories for individual flies that reflect what they eat, when they eat it, and how much they consume. We demonstrate that, for simple choices, the FLIC system performs as well as popular methods, and it provides an unprecedented opportunity to quantify novel components of feeding behavior, such as tasting events, time-dependent changes in food preference, and individual levels of motivation. Furthermore, FLIC experiments can persist indefinitely without disturbance. We highlight these strengths by

presenting data that reveal temporal dynamics of nutrient assessment and detailed circadian feeding patterns. A demo FLIC unit will be on display.

355C

The importance of a balanced diet: The effect of protein-to-carbohydrate ratio on body and organ size in *Drosophila*. Yuqing Zhu[1], Josephine R Masandika[1], Lily S Thorsen[1], Diego R Rojas-Toledo[1], Christen K Mirth[2], Alexander W Shingleton[1,3]. 1) Department of Biology, Lake Forest College, Lake Forest, IL; 2) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 3) Department of Zoology, Michigan State University, East Lansing, MI 48824, USA.

Proteins and carbohydrates are essential for organismal growth and development. While the individual effects of protein- and carbohydrate-deprivation on final body size have been well studied, what is less well understood is how the relative amount of proteins to carbohydrates in a diet affects growth. Here, we explore how the absolute amount and relative ratio of dietary proteins and carbohydrates affect final body size and body proportion, using *Drosophila* as a model organism. We reared flies on 24 different combinations of proteins and carbohydrates (four different food levels, each with six different protein-to-carbohydrate ratios), and measured the body parts of the resulting adults. As expected, a decrease in total amount of nutrients in a diet results in a decrease in final body and organ size. However, our data indicate an interaction between the quantity of proteins and carbohydrates on size, such that the effect of increasing carbohydrates depends on the quantity of proteins in a diet, and vice versa. Intriguingly, at low protein levels an increase in carbohydrates actually decreases body and organ size, whilst the opposite is true at high protein levels. Further, this interaction between proteins and carbohydrates is only detected in females, suggesting that it is a sex-specific effect. These data indicate that body size is not only influenced by the absolute amount of nutrients in a diet, but also how balanced the diet is.

356A

Reverse genetic screen for identification of non-autonomous growth signal(s) originating in the larval fat body. O. Carreño, M. Gallant, P. Gallant. Lehrstuhl für Biochemie and Molekularbiologie, University Würzburg, Germany.

Adult body size is determined during larval development in Drosophila, where the larval fat body acts as a signaling center in response to the animal's nutritional status. It activates growth pathways to send out diffusible signals that promote systemic growth. However, identy and regulation of these fat body-derived signals remain poorly understood. To identify genes involved in the production and release of these signals, we first selected ca. 1600 candidate genes based on their GO annotation, then systematically knocked them down in the fat body and used an enzyme-based assay to quantify the effects on the growth of imaginal discs. Positive hits were further characterized with independent RNAi lines and GAL4-drivers, and the consequences of knock-down on adult weight and wing size were documented. In the end, 3 % of the analysed genes were shown to act in the larval fat body to affect organismal growth. Almost half of these genes have been shown to be targets of the transcription factor Myc, pointing to a major role of Myc in this process. Most of these genes function in translation, mitochondrial activity, secretion/transport or gene regulation. Further analysis is expected to clarify the interaction between these pathways and lead to the identification of the secreted signaling molecule.

357B

Functional significance of the enzymatic and transcriptional co-regulator activities of dLipin. Michael Lehmann, Sandra Schmitt, Qiuyu Chen. Dept Biological Sci, Univ Arkansas, Fayetteville, AR.

In mammals and yeasts, proteins of the lipin family have been shown to act as both phosphatidate phosphatases and transcriptional co-regulators. Lipin's phosphatidate phosphatase (PAP) activity, which converts phosphatidic acid into diacylglycerol, constitutes an essential enzymatic step in the glycerol-3 phosphate pathway that leads to the synthesis of neutral fats (triacylglycerols). *Drosophila* lipin (dLipin) carries sequence motifs indicating that it has the same biochemical functions, but the biological roles of the PAP and transcriptional co-regulator activities remain largely unknown. To shed light on these roles, we generated a *Drosophila* mutant that lacks a functional PAP domain, but retains intact nuclear translocation and co-regulator motifs. We have begun to use this mutant and transgenic flies that express dLipin incapable of translocating into the nucleus (nuclear translocation-deficient dLipin, NTDdLipin) to study specific requirements for the PAP and co-regulator activities in development and metabolism. dLipin ΔPAP mutants die as first instar larvae, but can be rescued until later stages of development by expression of wild-type dLipin or NTDdLipin. However, while wild-type dLipin also rescues diminished starvation resistance of adult ΔPAP mutants, NTDdLipin does not. Moreover, expression of NTDdLipin diminishes starvation resistance in a heterozygous background, suggesting that it acts in a dominant-negative manner. Indeed, staining with dLipin antibody revealed that expression of NTDdLipin appears to prevent nuclear entry of endogenous dLipin. These data suggest that the transcriptional co-regulator activity of dLipin has an essential role under fasting conditions. However, it does not appear to be essential under normal, fed conditions, as suggested by the successful rescue of the lethality of dLipin mutants by NTDdLipin. Our data support a model in which dLipin coordinates physiological responses to changing nutritional conditions by shuttling between the cytoplasm and the nucleus. This model will be subject to further investigation.

358C

A role for tyrosine in mediating progression through oogenesis in *Drosophila*. Danielle Scheunemann, Tyler J. Halicek, Edward M. Blumenthal. Biological Sciences, Marquette University, Milwaukee, WI.

The reallocation of metabolic resources in response to changing environmental conditions is essential to survival. In female *Drosophila*, starvation activates apoptotic checkpoints in oogenesis and reduces the production of mature eggs. We found that

females homozygous for a mutation in *Tdc1*, which encodes the non-neuronal isoform of tyrosine decarboxylase, exhibited an extreme starvation-like phenotype. These females were sterile, and their ovaries absolutely lacked vitellogenic egg chambers. Previtellogenic egg chambers contained intact follicle cell (FC) layers but degenerating nurse cells and stained positively for activated caspase, consistent with activation of the mid-oogenesis apoptotic checkpoint. These phenotypes are unlikely due to an absence of tyramine, as tyramine receptor mutants are not female sterile. It has recently been reported in *C. elegans* that high levels of tyrosine result in the expression of starvation-dependent phenotypes in an AMPK-dependent manner (Ferguson et al., *PLoS Genetics*, 2013). We sought to determine whether a similar pathway in *Drosophila* could explain the female-sterile *Tdc1* phenotype. Consistent with this model, overexpression of constitutively-active *AMPKα* in the FCs resulted in a significant reduction in vitellogenic egg chambers compared to overexpression of wild-type *AMPKα*. Conversely, overexpression of kinase-dead *AMPKα* resulted in increased oogenesis. Experiments to overexpress these *AMPKα* mutants in the female germline are in progress. We also tested the possibility that tyrosine is an endogenous signal linking starvation to a reduction in oogenesis. Two lines of evidence argue against this model. First, the expression of two tyrosine-metabolizing enzymes, *Tdc1* and *CG1461* (tyrosine aminotransferase) was unchanged following 4-5 days of protein starvation. Second, global overexpression of *Tdc1*, which would be expected to reduce the level of tyrosine in the hemolymph, had no effect on the starvation-induced reduction in vitellogenesis. Thus it appears that elevated tyrosine can induce a starvation-like arrest of oogenesis but is not itself the physiological starvation signal. *Supported by an NSF REU site, DBI-1156569*.

359A

The *Drosophila* Estrogen-Related Receptor acts as a nutrient sensor to coordinate larval growth with nutrient availability. Maria C Sterrett, Samantha L St. Clair, Jason M Tennessen. Indiana University, Bloomington, IN.

All growth during the *Drosophila* life cycle is restricted to larval development, when animals increase their body size ~200-fold over the course of four days. To support this exponential growth, larvae rely on aerobic glycolysis, a unique metabolic program ideally suited to synthesize biomolecules from carbohydrates. Our previous work demonstrated that aerobic glycolysis is transcriptionally-activated during embryogenesis, when the *Drosophila* ortholog of the Estrogen-Related Receptor (ERR) class of nuclear receptors coordinately up-regulates genes involved in glycolysis, the pentose phosphate pathway, and lactate production. We have discovered that dERR activity is not restricted to embryogenesis; rather, dERR also promotes aerobic glycolysis during larval development. Our preliminary analyses demonstrate that dERR protein is expressed in key metabolic tissues, including the fat body, intestine, and muscle. This expression pattern suggests that dERR coordinates glucose-derived biosynthesis with growth conditions. Consistent with this model, we have discovered that the dERR ligand-binding domain (LBD) is activated when larvae are fed a yeast-based diet but not sugar-only or starvation media. Furthermore, the addition of insulin to larval organ cultures fails to activate the dERR LBD, suggesting that dERR represents a novel mechanism for linking dietary conditions with sugar metabolism. We have also determined that dERR is covalently modified under starvation conditions, suggesting that a nutrient-sensitive enzyme controls dERR activity. Finally, we have demonstrated that many dERR target genes are transcriptionally down-regulated upon starvation, indicating that diet-induced changes in dERR activation are functionally significant. Overall, our studies indicate that dERR promotes aerobic glycolysis in response to dietary compounds and suggest that mammalian ERRs also act as nutrient sensors that coordinate biosynthesis with rapid growth.

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360B

Juvenile Hormone regulates body proportion in *Drosophila*. Christopher D Mirque[1], James J Haney[1], Lauren M Lyon[1], Thomas Flatt[2], Christen K Mirth[3], Alexander W Shingleton[1,4]. 1) Department of Biology, Lake Forest College, Lake Forest, IL 60045, USA; 2) Department of Ecology and Evolution, University of Lausanne, UNIL Sorge, Biophore, CH-1015, Switzerland; 3) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 4) Department of Zoology, Michigan State University, East Lansing, MI 48824, USA.

The developmental regulation of body proportion is a fundamental yet poorly understood phenomenon that ensures the size of each organ is appropriate to the size of the body as a whole. The regulation of body proportion ultimately relies on the inter-organ coordination of growth rate and duration, and previous studies indicate that the developmental mechanisms that regulate relative organ size overlap with the mechanisms that regulate overall body size. We have previously identified Juvenile Hormone (JH) as a novel regulator of body size in *Drosophila*. Here we demonstrate that it is also a regulator of body proportion. Flies mutant for the putative JH receptor *Met* show a reduction in overall body size, but have proportionally larger wings. Knock-down of *Met* expression in the imaginal discs does not influence body size or proportion, suggesting that the effect of JH on body proportion is not organ-autonomous. In contrast, knock-down of *Met* in the prothoracic gland alone does influence body size and proportion, implicating ecdysone in the regulation of body proportion in *Met* mutant flies. This hypothesis is supported by the observation that flies that lack JH have elevated levels of ecdysone signaling, while flies with up-regulated ecdysone synthesis, like *Met* mutants, have smaller bodies but proportionally larger wings. Collectively these data suggest that JH regulates body proportion in part via ecdysone signaling.

361C

A novel function of the hippo-pathway member, *warts*, in modulating organism size of *drosophila*. Morten E. Møller[1], E. Thomas Danielsen[1], Michael B. O'Connor[2], Kim F. Rewitz[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota, USA.

Pulses of the steroid-hormone ecdysone, drives the developmental progression through the larval stages and initiation of metamorphosis that insures the transition to adulthood in *Drosophila*. Ecdysone is synthesized from dietary cholesterol, though several enzymatic steps in the prothoracic gland (PG) and is released systemically. Ecdysone is transformed to its active form 20-

hydroxyecdysone in peripheral tissues. Besides its role in dictating developmental transitions, ecdysone is tightly linked to the metabolism and growth of the larva. During larval development, basal levels of ecdysone affect growth through its interaction with insulin signaling. We have conducted an *in-vivo* genome-wide RNAi screen targeting the PG, using the Gal4/UAS system, to identify the genes that are essential for its steroidgenic activity. Among the genes identified in this screen, we found that PG-specific loss of the tumor-suppressor gene *warts* (*wts*) in the hippo pathway, results in larval overgrowth and increase in final body size. In Drosophila final body size is determined by the growth during development since growth is restricted to the larval stages. The timing of pupariation therefore determines the duration of the growth period. Larvae with reduced expression of *wts* in the PG pupariated on time, suggesting that loss of *wts* accelerated growth during the larval stages without affecting the duration of growth. Furthermore, we found that knock down of *wts* in the PG affects ecdysone levels, suggesting a mechanism whereby decreased *wts* expression in the PG leads to an overall overgrowth. *wts* and the other members of the hippo pathway are key-regulators of tissue and organ size. Our data shows that *wts* via control of ecdysone biosynthesis in the PG is important in regulating organismal size. In conclusion, our data show a novel function of *wts* in regulating final body size, in addition to its known role in determining tissue and organ growth.

362A

Autophagy in prothoracic gland controls developmental timing in Drosophila melanogaster. Xueyang Pan[1,2], Michael O'Connor[1]. 1) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Molecular, Cellular, Developmental Biology and Genetics Program, University of Minnesota, Minneapolis, MN.

During development of Drosophila melanogaster, availability of nutrient impacts the timing of metamorphosis in a body weight-dependent fashion. During L3 larva stage, starvation before larva reaching a body weight threshold termed critical weight (CW) results in developmental arrest before metamorphosis, while fasting after attainment of CW does not cause any developmental delay. The determination of CW checkpoint effectively prevents animals from precocious metamorphosis that ends up with pupal lethality. In previous studies insulin and TOR pathway have been shown to couple the nutrient input and timing of metamorphosis in prothoracic gland (PG), the endocrine organ synthesizing molting hormone ecdysone, but detailed mechanism is not yet clarified. Macroautophagy (hereafter autophagy) is a downstream event of TOR pathway and commonly responses to nutrient insufficiency in various models. Therefore, in this study we hypothesized a potential function of autophagy on developmental timing control under food restriction condition. We found that starvation markedly stimulates autophagy in PG tissue during pre-CW but not post-CW stage, and suppression of autophagy causes abnormal pupariation when larvae are starved before reaching CW. The autophagy process in PG is controlled by TOR pathway and requires most autophagy related genes, which are consistent with the process observed in other models. However, the autophagosomes in PG do not fuse with lysosome, despite of their high acidity indicated by quenching of GFP signal of the Atg8a marker. Since autophagosomes commonly deliver their inside contents for degradation, and simultaneously get acidified, by fusing with lysosome, autophagy in PG may exert a non-canonical and possibly non-degradative function. Taken together, our results suggest the role of autophagy in PG in determination of CW checkpoint and metamorphosis control during fasting condition, yet its functioning mechanism remains to be studied.

363B

A genome-wide *in vivo* RNAi screen in *Drosophila* identifies regulators of cholesterol-dependent steroid production. Morten Moeller[1], E. Thomas Danielsen[1], Naoki Yamanaka[2], Kirst King-Jones[3], Michael O'Connor[4], **Kim Rewitz**[1]. 1) Biology, University of Copenhagen, Copenhagen, Copenhagen, Denmark; 2) Entomology, University of California, Riverside, USA; 3) Biological Sciences, University of Alberta, Alberta, Canada; 4) Genetics, Cell biology and Development, University of Minnesota, Minneapolis, USA.

Steroid hormones are signal molecules synthesized from cholesterol that regulate a variety of processes during embryogenesis, postembryonic development and reproduction. Although steroid synthesis in endocrine cells requires uptake of cholesterol, the mechanisms regulating cholesterol uptake, storage and availability for steroid production are poorly understood. We have performed a genome-wide *in vivo* RNAi screen to uncover the genes required for steroid production in the endocrine steroid-producing cells of *Drosophila*. Using this approach, we reduced the expression of 12,600 genes in the steroid-producing prothoracic gland (PG) cells to investigate their potential role in steroid biosynthesis. Cholesterol is a low density lipoprotein (LDL)-derived lipid taken up by cells through endocytosis and stored as cholesteryl ester in lipid droplets. To identify novel genes involved in cholesterol uptake and transportation, we performed a secondary screen to analyze which of the genes identified in our primary screen for steroidogenesis are specifically involved in cholesterol transport. In this screen we identified 25 novel genes that seem to be involved in regulating cholesterol uptake and trafficking for steroid production. To investigate dysregulation of cholesterol accumulation, we used Coherent Anti-Stokes Raman Scattering (CARS) microscopy, which can selectively detect lipids including cholesterol and cholesteryl esters. Loss of genes identified in our screen caused defects in the formation of cholesterol-rich lipid droplets indicating that the genes are involved in uptake, trafficking and storage of cholesterol. Many of these novel genes have conserved human homologs that have been associated with diseases that involve dysregulation of cholesterol homeostasis and steroid signaling.

364C

A novel ecdysteroidogenic gene *noppera-bo* encoding glutathione *S*-transferase regulates cholesterol behavior. Enya Sora[1], Yuko Shimada-Niwa[1], Fumihiko Igarashi[2], Masatoshi Iga[3], Hiroshi Kataoka[3], Tetsuro Shinoda[4], Ryusuke Niwa[1,5]. 1) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, ibaraki, Japan; 2) Biotechnology Research Center, Toyama Prefectural University, Toyama, Japan; 3) Graduate School of Frontier Sciences, University of Tokyo, Japan; 4) Division of Insect Science, National Institute of Agrobiological Sciences, Japan; 5) PRESTO, JST, Japan.

In insects, ecdysteroids regulate many physiological events such as molting, metamorphosis and oogenesis. In larval stages, ecdysteroids are synthesized from dietary cholesterol in the specialized endocrine organ so called prothoracic gland (PG). In the last decade, a number of ecdysteroidogenic enzymes including the Rieske oxygenase, the short chain dehydrogenase/reductase and several cytochrome P450 monooxygenases, have been identified and characterized at the molecular level. Besides these identified enzymes, here we report a novel ecdysteroidogenic enzyme, Noppera-bo (Nobo), which belongs to the glutathione *S*-transferase family. *nobo* is predominantly expressed in the PG and adult ovary. We generated a *nobo* knockout mutant, which displayed embryonic lethality and a naked cuticle structure. These phenotypes are observed in other ecdysteoridogenic enzyme gene mutants. In addition, the PG-specific *nobo* RNAi larvae displayed an arrested phenotype and reduced 20-hydroxyecdysone (20E) titers. Importantly, both embryonic and larval phenotypes were rescued by 20E administration. These results suggest that *nobo* is essential for ecdysteroid biosynthesis. The larval lethality of *nobo* RNAi animals was rescued by feeding not only 20E but also cholesterol. Furthermore, *nobo* RNAi caused the abnormal cholesterol accumulation in the PG. Considering that cholesterol is the most upstream material for ecdysteroid biosynthesis in the PG, we concluded that *nobo* plays a crucial role in cholesterol transport or metabolism in the PG. Further analyses investigating the molecular functions of *nobo* in ecdysteroid biosynthesis will be presented.

365A

Regulation of enterocyte cell death by dMyc modulates intestinal barrier function and lifespan in *D. melanogaster*. Kazutaka Akagi, Subhash Katewa, Kenneth Wilson, Subir Kapuria, Amit Sharma, Arshad Ayyaz, Heinrich Jasper, Pankaj Kapahi. Buck Inst Research Aging, Novato, CA.

Loss of gut integrity has been associated with various human diseases like inflammatory bowel disease, however, the mechanisms which lead to loss of barrier function remain poorly understood. Here we identify a critical role for intestinal cell survival mediated by dMyc in the regulation of intestinal barrier function in *Drosophila melanogaster*. We demonstrate that dietary restriction (DR) slows the age-related decline in intestinal integrity by reducing enterocytes cell death through up-regulation of *dmyc* in the intestinal epithelium. Reduction of *dmyc* in gut enterocytes enhances cell death through JNK signaling and leads to enhanced gut permeability. This also increases vulnerability to pathogenic bacteria and abrogates DR-mediated lifespan extension due to systemic infection. The effects of loss of *dmyc*in the gut can be rescued by inhibiting Caspase activity or antibiotic treatment. Furthermore, knockdown of *caspase-9* (*dronc*) or overexpression of *dmyc* in the intestinal cells is sufficient to extend lifespan under rich nutrient conditions. We propose that dMyc in gut enterocytes plays a critical role in extending organismal lifespan by maintaining intestinal barrier function in response to nutritional changes.

366B

Lifespan extension by ginseng berry extract and its active component syringaresinol. Kyung-Jin Min[1], Shin-Hae Lee[1], Hye-Yeon Lee[1], Si-Young Cho[2], Ju-Won Kim[2], Sang Joon Lee[2]. 1) Department of Biological Sciences, Inha University, Incheon, South Korea; 2) R&D Center, Amorepacific Corporation, Gyeonggi-do, South Korea.

Ginseng is well known for its beneficial effects for many aspects of health. So far, the root of ginseng has been extensively studied, but recent studies indicate that the fruit of ginseng, ginseng berry is also useful and contains more amounts of some ginsenosides like Re, Rb2 and Rd. We investigated the anti-aging effects of ginseng berry using flies and worms. Ginseng berry extract extended the lifespan of flies and worms. The active component of lifespan extension by ginseng berry was found to be syringaresinol. Syringaresinol treatment increased the lifespan of flies and worms and the effect was dependent on FOXO. Syringaresinol seems to activate FOXO by direct binding to forkhead domain of FOXO. The effect of lifespan extension by syringaresinol was not additive to the effect of dietary restriction both in flies and worms and metabolite assay also showed similar metabolite patterns between dietary restricted and syringaresinol treatment groups. Overall, the positive effects of ginseng berry and its active component, syringaresinol demonstrate a potential broad applicability of ginseng berry as anti-aging treatment.

367C

Mitonuclear AxGxE: Genetic and dietary interactions are as important as single factors in explaining Drosophila lifespan and healthspan. David Rand, C.-T. Zhu, J. Mossman, J. Santiago, A. Spierer, L Biancani, T. Devlin, J. Dewey, B. Frankin, M. McAteer, Z. Pataki, C. HalE-Phillips, D Yoon. Ecology & Evolutionary Biol, Brown Univ, Providence, RI.

The role of mitochondria in the extension of longevity by dietary restriction (DR) is widely recognized but poorly understood. To dissect this complexity we have constructed a novel panel of 72 mitonuclear genotypes to map fitness and longevity phenotypes on different diets and hypoxic environments. We have placed 6 different mtDNAs (3 from D. melanogaster and 3 from D. simulans) on to 12 sequenced nuclear genetic backgrounds of D. melanogaster from the DGRP panel (Mackay et al. 2012, Nature) that vary in longevity and starvation resistance. Development time varies among all 72 genotypes, and mtDNA x nuclear genetic interactions are as important as the main effect of either mtDNA or nuclear genotype. Using a subset of 12 mitonuclear genotypes we quantified the joint impact of mtDNA genotype, nuclear genotype and protein- and sugar-restriction diets on several measures of healthspan at two ages: starvation resistance, climbing speed, body weight, protein, triacylglyceride and glycogen content. As expected, older flies show reduced starvation resistance and climbing speed, but these declines are strongly dependent on mtDNA x nuclear genotype combinations. Clear interactions between specific DGRP nuclear genotypes and mtDNAs show reversals of survivorship on high-protein vs. high-sugar diets, with one mtDNA resulting in extended starvation resistance. These mito x nuclear x diet interactions are pervasive and are sufficiently pronounced to permit forward genetic mapping of the interacting mtDNA and nuclear loci, as we have reported (Meiklejohn et al. 2013 PLoS Genetics; Zhu et al. 2014 PLoS Genetics). Overall, these studies show an age-specific modification of mitonuclear genetic

interactions that regulate diet-mediated changes to healthspan (an age x genotype x environment interaction, or AxGxE). Because these interactions terms are as significant in the biology of aging as single factors, efforts to map pathways controlling DR and CR should be done in multiple genetic backgrounds to distinguish general from context specific effects. .

368A

Analysis of the cSOD-Null Mutant Phenotypes of *Drosophila melanogaster* Across a Suite of Genetic Backgrounds. Courtney E Lessel[1], Thomas J S Merritt[1], Tony L Parkes[2]. 1) Biology Dept, Laurentian University, Sudbury ON; 2) Biology and Chemistry Dept, Nipissing University, North Bay ON.

Mutations often have drastically different effects in different genetic backgrounds. Understanding a genes biological function, then, requires an understanding of its interaction with genetic diversity. Cytosolic superoxide dismutase (cSOD) catalyzes the dismutation of the superoxide radical into hydrogen peroxide and oxygen. The superoxide radical is produced as a metabolic by-product and functions in cell signalling and regulation. Superoxide can become a source of oxidative stress if its concentration exceeds cellular control. *Drosophila melanogaster* that lack functional cSOD (cSOD-null) exhibit a suite of phenotypes including decreased longevity, hypersensitivity to oxidative stress, and accelerated impairment of adult locomotor function. Recently, large metabolomic differences have also been observed between *cSod-null* and *cSod+ D. melanogaster*. However, *cSod-null* phenotypes have primarily been classified using one allele, $cSod^{n108}$, and its associated genetic background, leaving the question about the influence of genetic background on *cSod-null* mutant phenotypes unanswered. We are using bio- and enzyme-assays to quantify the influence of genetic background on the cSod-null mutant phenotype. Preliminary results indicate that some, but not all, phenotypes respond to background suggesting that sensitivity may reflect differences in the underlying biology. .

369B

The Role of miR-310s in ovarian soma in response to dietary conditions. Ibrahim Omer Cicek, Halyna R. Shcherbata. Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

miRNAs are short noncoding RNA molecules that regulate gene expression and have been shown to act on diverse cellular and physiological processes in their response to environmental stress conditions. We found by a proteomics approach (SILAC-Mass Spectrometry) that the recently evolved *mir-310s* complex plays an important role in the regulation of the genes sensitive to nutrient restriction. To determine one of the roles of the *mir-310s* more specifically, we focused on oogenesis and characterized epithelial defects that are enhanced due to nutritional stress conditions. The deletion of the *miR-310s*resulted in the fused egg chamber, multilayered follicular epithelium, and stalk and phenotypes. The *miR-310s* are expressed in the somatic cells at the anterior tip of the germarium, at the stalk, and at the late follicular epithelium. By the conserved seed sequences of the *mir-310s* it is predicted that three elements of the highly evolutionary conserved Hedgehog (Hh) pathway (*Rab23, tramtrack (ttk),* and*Hormone receptor-like in 96 (DHR96)*) are putative targets of the *mir-310s*. We have demonstrated that these three genes are targeted by the *mir-310s* in vitro and in vivo. Furthermore, we acquired data suggesting the the *Rab23* functions in the Hh pathway paralleling its vertebrate homologue with the difference in *Drosophila*, were it acts cell-autonomously in the Hh signal-sending cells. It is well known that Hh signaling regulates the generation of the follicular epithelium and cooperates with TGF-β, Wg, and JAK/STAT signaling to control follicle stem cell proliferation that also is extremely sensitive to growth factors. Our data imply that the strength of Hh signaling in the ovary is tightly regulated by *miR-310s* in response to diet.

370C

How does diet regulate lifespan? Organ-specific, Tor-dependent transcriptomic responses to essential amino acids. Adam Dobson[1], Mingyao Yang[1,2], Xiaoli He[1], Eric Blanc[3], Matthew Piper[1]. 1) Institute of Healthy Ageing, Department of Genetics, Evolution and Environment, University College London, London, UK; 2) Institute of Animal Genetics and Breeding, Sichuan Agricultural University, Chengdu, Sichuan, China; 3) Medical Research Council (MRC) Centre for Developmental Neurobiology, King's College London, London, UK.

Dietary essential amino acids (EAAs) regulate a suite of physiological, life-history and ageing-related traits. Indeed, the lifespan-extending effects of dietary restriction can be attributed to the EAA content of the diet. We have recently shown that effects of EAAs on lifespan can be specifically blocked by pharmacological inhibition of the Tor pathway, by feeding flies with rapamycin, offering a precise set of tools with which to dissect the molecular events underlying lifespan regulationby dietary restriction. We describe the transcriptomic effects of EAA enrichment in brains, fat bodies, guts, ovaries, thoraces and whole flies, and use rapamycin to determine the proportion of these transcriptomic changes that are Tor-mediated and thus associated with longevity assurance. Both the extent to which the transcriptome is affected by EAAs and how much of this effect is Tor-mediated are highly organ-specific. Furthermore, both the identity of the genes affected by EAAs and rapamycin and their known functions are also organ-specific. Targets of EAAs/Tor include some of known significance for ageing and stress resistance, metabolic enzymes, and further fitness-relevant targets of EAAs/Tor e.g. immunity. Finally we predict transcription factors for the regulation of EAA/Tor-dependent effects. Thus, our study systematically characterises mechanisms associated with diet-dependent changes in metabolism and physiology, and identifies new gene targets for modulating ageing without the costly side effects of wholesale dietary restriction.

371A

Absence of mitochondrial SOD2 in *Drosophila* induces a novel peroxidase, which plays an essential role during adult wing maturation. Dondra Bailey, Sanjay Nag, Mohammed Basar, **Atanu Duttaroy**. Dept Biol, Howard Univ, Washington, DC.

SOD2 (superoxide dismutase 2) is a nuclear encoded protein, which catalyzes the conversion of superoxide radicals into hydrogen peroxide in the mitochondria. A null mutant for the *Sod2* gene, *Sod2*^{n283}survives a very short adult life span like the *Sod2* KO mice. Should this high flux of mitochondrial ROS in *Sod2*^{n283} facilitate the activation of novel gene(s) most likely through retrograde mechanism? Gene array analysis revealed various transcriptomes are altered significantly in their expression pattern in the *Sod2*^{n283}. Notably among them is the *Drosophila* gene *CG5873,* which was upregulated ~40 fold in*Sod2*^{n283}. CG5873 carries a highly conserved domain classified as the animal peroxidase superfamily. Interestingly, both the insertion mutant as well as ubiquitous ablation of *CG5873* with RNA RNAi shows a collapsed and wrinkled wing phenotype in adults. Our analysis with early wing specific GAL4 drivers, revealed no associated defects in wing morphology when combined with the *CG5873IR,* which led us to conclude that CG5873 is not required during wing development. On the other hand, ablation of *CG5873* during adult wing maturation shows collapsed wing phenotype, suggesting the requirement of this peroxidase function during tissue remodeling process of adult wing maturation. .

372B

The FIT in fat: Mating behavior and metabolism are influenced by fat body FIT expression and secretion into hemolymph. Hina Iftikhar, Ginger Carney. Biology, Texas A&M University, College Station, TX.

Vertebrate adipose tissue modulates central nervous system (CNS) activity via the secretion of hormonal factors known as adipokines. These factors can affect the development of the neural circuitry as well as the response of the CNS to environmental changes. Insect fat body functions analogously to vertebrate adipose and hepatic tissues. Fat body serves as a repository for lipid and glycogen stores, but it also is an endocrine gland that produces and releases factors into the hemolymph (insect plasma). Our lab's previous work indicated that sexual and social experience affect expression of a fat body-expressed gene, *female-specific independent of transformer (fit)*. Unpublished work suggests that *fit* modulates both courtship behavior and starvation responses. The predicted FIT protein has a signal sequence, indicating that the protein may be secreted by the fat body into hemolymph. We hypothesized that circulating FIT signals to the CNS to influence behavior and metabolism. To determine if FIT is secreted, we developed an anti-FIT antiserum and tested it in *D. melanogaster* via Western blotting. Since *fit* has female-biased expression, we first tested female tissues for FIT protein. We detected FIT in female whole bodies and hemolymph. *fit* transcripts previously were detected predominantly in head fat body and at much lower levels in the brain. Although we also detected FIT in heads, expression was lost when we compared FIT expression separately in brains and head carcasses (contain head fat body). One explanation for not detecting FIT in head carcasses is that the rate of protein translation does not match the rate of transcription for the gene. It could also be that FIT is quickly secreted into the hemolymph and is not present in fat body in detectable amounts. Determining where FIT is located can help in understanding which tissues are influenced by this fat-expressed and secreted protein that modulates mating and metabolism.

373C

Mitochondrial knockdown using tissue specific expression of mitochondrially-targeted restriction enzymes against mtDNA as a model for the decline in organismal performance. Adam N. Spierer, A. Pascal, R. Mabeza, David M. Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

Mitochondrial biogenesis and replication requires ~1200 genes encoded in the nucleus and 37 genes encoded in the mitochondrial genome (mtDNA). The replication of mitochondria takes place independent of the cell cycle, but requires the import of many nuclear encoded proteins that must interact with mtDNA-encoded proteins to produce a functioning organelle. While most tissues in adult Drosophila are post-mitotic, they require continued mitochondrial activity to persist. The turnover of mitochondria in different tissues, and the impact of this process on organismal performance is poorly understood. To model this dynamic process, we expressed a restriction enzyme against mtDNA (mitoRE) during development and in different adult tissues, using the GAL4/UAS and Geneswtich systems. Extending work from the O'Farrell lab (Xu et al, 2008, Ma et al. 2014), we show that constitutive expression of the mitoRE in the germ line using a nanos driver results in viable but sterile adults, indicating that intact mtDNAs are required for the survival of germ line tissue leading to fertility. Constitutive expression of the mitoRE in the nervous system using the ELAV driver is lethal, indicating that mitochondria in neuronal tissue are required for viability. Knockdown of mtDNA in adult muscle and nervous system, using the MHC and ELAV geneswitch systems, respectively, resulted in decreased locomotor performance, which was sensitive to dose and the age of the fly. These observations indicate that mitochondrial biogenesis is required for the maintenance of cellular function implying a dynamic process of mitochondrial turnover in tissues that are no longer mitotically active. Using the time-dependent decline in performance of adult flies expressing the mitoRE in different tissues, we develop a model of mitochondrial turnover with relevance to aging and neurodegenerative diseases.

374A

Endurance exercise and selective breeding for longevity extend *Drosophila* healthspan by overlapping mechanisms. Alyson Sujkowski[1], Brian Bazzell[3], Kylie Carpenter[3], Robert Arking[2], Robert Wessells[1]. 1) Wayne State School of Medicine, Detroit, MI; 2) Wayne State University, Detroit, MI; 3) Univ of Michigan, Ann Arbor, MI.

Endurance exercise has emerged as a powerful intervention to extend healthy physiology into advanced age. Normal, age-associated declines in organ structure and function are alleviated in endurance-trained individuals. Long-term exercise also reduces the incidence of age-related pathologies in humans and in model organisms. Despite these evident benefits, the genetic pathways required for exercise interventions to achieve these effects are still relatively poorly understood. Here, we compare the effects of endurance training on *Drosophila melanogaster* to the effects of selective breeding for longevity. We find that both selective breeding and endurance training increase endurance, cardiac performance, running speed, flying height, and levels of autophagy in adipose tissue. Microarrays

indicate that 73% of transcriptional changes found in flies selectively bred for longevity are also found in flies subjected to three weeks of exercise training. Both endurance training and longevity selection upregulate folate biosynthesis, stress defense and lipid metabolism. Conversely, both interventions downregulate multiple carbohydrate metabolism pathways. *methuselah-like 3,* a *Drosophila* G-protein coupled receptor thought to be involved in stress resistance and longevity, was also transcriptionally reduced in both groups. Altering global *mthl3* expression in adult flies was able to reproduce a subset of the phenotypes shared between endurance trained and longevity selected flies. These results provide support for endurance exercise as a broadly acting anti-aging intervention and confirm that exercise training acts in part by targeting longevity assurance pathways. In addition, these results identify the *mthl3* gene as a potential target of exercise training.

375B

The nuclear receptor dHNF4 coordinates a transition towards oxidative phosphorylation and glucose-stimulated insulin secretion at the onset of adulthood. William E. Barry, Carl S. Thummel. Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

Mutations in the nuclear receptor *HNF4α* were identified as the cause of Maturity Onset Diabetes of the Young 1 (MODY1) nearly two decades ago. Despite intensive study, however, an animal model of MODY1 is still lacking, along with an understanding of how HNF4α regulates glucose homeostasis. Here we report that loss of *Drosophila dHNF4* faithfully recapitulates the hallmark symptoms of MODY1 including adult-onset hyperglycemia, glucose intolerance and impaired glucose-stimulated insulin secretion. dHNF4 functions in both the fat body and insulin producing cells to regulate a developmental switch in glucose homeostasis at the transition to adulthood. dHNF4 mediates this process, in part, through an unexpected role in mitochondria, where it associates with the promoter region of mtDNA to increase expression of electron transport chain genes and support a shift towards oxidative phosphorylation and glucose stimulated insulin secretion. In addition, we find that expression of the adult-specific glycolytic gene, *HexC*, is dependent on dHNF4, and its expression is required in the fat body to maintain euglycemia. Taken together, these findings establish the first animal model of MODY1 and uncover a novel role for dHNF4 in mitochondrial-encoded gene expression. In addition, we demonstrate that dHNF4 contributes to a metabolic switch at the pupal-adult transition that promotes glucose oxidation, supporting the energetic needs of the mature animal.

376C

Elucidating the role of CyclinB3 in mediating lifespan extending effects of reduced insulin signaling in *Drosophila*. Ekin Bolukbasi[1], Jennifer Regan[1], Linda Partridge[1,2]. 1) Insitute of Healthy Ageing, UCL, London, United Kingdom; 2) Max Planck Institute for Biology of Ageing, Cologne Germany.

Ageing is a major risk factor for the most common lethal and debilitating conditions in the developed world, including cancer, cardiovascular and neurodegenerative diseases. A key discovery of recent years has been that mutations in single genes can extend the healthy lifespan of laboratory animals. Nutrient sensing pathways, such as Target of Rapamycin and insulin/insulin-like growth factor signaling (IIS), were shown to be important signalling networks that can be altered to extend lifespan in diverse model organisms. Forkhead Box-O (FOXO) proteins are an important and evolutionarily conserved family of transcription factors that are directly regulated by the IIS pathway. In *Drosophila*, the lifespan extension observed upon IIS reduction requires *Drosophila foxo (dfoxo)* and the restricted overexpression of*dfoxo* leads to an increased longevity highlighting its key role during the regulation of ageing. However, the downstream target genes of dFOXO involved in mediating its lifespan extending effects still remain elusive. To understand how dFOXO alters gene expression to extend lifespan, recent work from our laboratory uncovered, at a genome wide scale, gene targets of dFOXO that respond to reduction in IIS activity. One interesting candidate identified by this study is *cyclinB3 (cycB3),* which is involved in the regulation of cell cycle progression. Interestingly, ubiquitous over-expression of *cycB3* results in increased longevity in adult *Drosophila*. In order to determine which tissues might be mediating this lifespan extension, we overexpressed *cycB3* in a tissue specific manner and identified adult neurons as the key tissue responsible for the observed longevity phenotype. To complement lifespan assays, we examined the effect of *cycB3* overexpression on progression of cell cycle in larval and adult tissues. Our results suggest an arrest of endoreplication upon the overexpression of *cycB3*. The link between endoreplication, neuronal tissue and longevity is intriguing, and we are currently testing this in the ageing brain.

377A

Tissue-specific effects of insulin signaling on female attractiveness. Tatyana Fedina, Scott Pletcher. Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI.

We had previously found that ubiquitous upregulation of insulin signaling (IS) in adult females increases their attractiveness, while IS downregulation makes them less attractive to males than corresponding controls in two-choice behavioral assays. In these assays female attractiveness is driven solely by pheromones, which are secreted as cuticular hydrocarbons (CHCs) and are transcriptionally regulated by IS. Since we originally hypothesized that IS activity (via CHCs) is used by males to assess female reproductive potential, this study investigates whether ovaries and interacting tissues are responsible for relaying IS status. Several tissue-specific and inducible or adult-expressing Gal4 drivers together with UAS-Pten-OX and UAS-InR-OX transgenes were used to downregulate and upregulate IS, respectively. Reproducing the effects of ubiquitous IS manipulations on female attractiveness with tissue-specific manipulations would suggest involvement of that tissue. Manipulation of insulin signaling in CHC-producing cells, oenocytes, did not affect female attractiveness, suggesting that the effects of ubiquitous IS manipulations are largely cell non-autonomous. Interestingly, IS downregulation in adult fat body (FB) increased female attractiveness, ovary size, and fecundity, while IS upregulation produced

somewhat opposite response. One potential explanation for this is IS competitive regulation between ovaries and FB. It agrees with previously shown suppression of systemic IS in response to overexpression of FB-specific insulin, dilp6. To investigate whether ovarian activity is required for IS effects on attractiveness, we tested non-vitellogenic sterile females carrying OvoD1 mutation together with geneswitchTub5-Gal4 and either UAS-Pten-OX or UAS-InR-OX. Unlike females possessing normal ovaries, these OvoD1 females were not differentially attractive to males upon IS activation/inhibition. Therefore, current data suggest a model whereby ovaries compete with FB for nutrients and mediate IS effects on attractiveness. We are in the process of further dissecting which ovarian tissues and upstream/downstream factors are responsible for relaying IS activity into female attractiveness, fecundity, and CHC composition.

378B

The sex determination gene *transformer* controls male-female differences in growth. Elizabeth Rideout[1,2], Savraj Grewal[1]. 1) Clark H. Smith Brain Tumour Center, Dept. Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada; 2) Dept. Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada.

Male and female *Drosophila* exhibit differences in behaviour, sexual differentiation and body size. The genetic interactions that control sexually dimorphic behaviours and differentiation have been well studied. However, the mechanisms underlying sex differences in body size remain unclear. Here we demonstrate a novel role for the sex determination gene *transformer* (*tra*) in the regulation of cell and body size. Normally, Tra is expressed exclusively in females, where it determines most aspects of female sexual behaviour and differentiation. Females lacking Tra function are masculinized in appearance and behaviour. Ectopic expression of Tra in males, on the other hand, leads to female differentiation and behaviour. Whether Tra also affects sex differences in body size remains largely unknown. We show that loss of Tra function in females significantly decreases body size. Conversely, expression of Tra in males significantly increases body size. These effects of Tra on body size are mediated by both cell-autonomous and non cell-autonomous mechanisms. In female cells, loss of Tra function leads to cell-autonomous decrease in cell size. In males, Tra expression leads to a cell autonomous increase in size. Remarkably, loss of Tra function specifically in the female fat body, the main larval endocrine organ, leads to a decrease in overall body size. Previous studies have demonstrated that the fat body plays an important endocrine role in coupling dietary nutrient input to secretion of brain-derived insulin-like peptides (Ilps), and control of systemic insulin signaling and body growth. Our data suggests Tra activity in the fat body may similarly regulate the production/release of Ilps to control sex differences in body size. Indeed, we found that Tra's effects on cell and body size require insulin signaling, and that females have higher levels of insulin signaling than males. Together, these findings suggest that female body size is larger than male body size due to the stimulation of systemic insulin signaling by Tra activity in the fat body..

379C

Connections between the effects of various chemicals on the development of *Drosophila melanogaster* and *Homo sapiens*. Amy Brenner. Biology Department, Olivet Nazarene University, Bourbonnais, IL.

This study, funded by the Elbert Pence and Fanny Boyce Grant in the biology department at Olivet Nazarene University, attempted to determine the relationship between effects of four chemicals on*Drosophila melanogaster* and humans. Utilizing previous human studies, effects of theobromine, caffeine, ethylene glycol, and ammonia on *D. melanogaster* were observed in P generation and F_1development, to predict effects on humans. Triplicate trials were conducted with a dose range interval of 0.1 for three chemicals. The dose range interval for ammonia was 0.025. The control group contained no added chemicals. Observations were documented daily, noting mortality of P generation, progression of development, and F_1 viability. First, chi-square tests were completed indicating differences in toxicity of chemicals on P generation and F_1 viability. Second, multiple correlation tests were used to analyze overall toxicity of each chemical and F_1 viability. Third, a one-way between subjects analysis of variance (ANOVA) was used to analyze effects of each chemical on *D. melanogaster* development. Fourth, a Tukey post hoc procedure was implemented to determine which of the chemicals had a significant effect on number of days in each developmental stage. Fifth, correlation tests were run between concentrations of all chemicals combined and overall effect on developmental stages. Finally, based on these results, further correlation tests were run to determine individual chemicals' effects. Inferences of human effects were formed based on results of this and previous studies.

380A

Metabolic and genetic implications of survival in anoxia. Jacob Campbell, Jon Harrison. Arizona State University, Tempe, AZ.

Anoxia or severe hypoxia is a fundamental component of pathologies associated with heart disease, stroke and many other human pathologies; yet we still lack a fundamental understanding of how anoxia/severe hypoxia kills and the mechanisms responsible for the wide variation in anoxia tolerance across animals. *Drosophila* larvae feed on the yeasts of rotting fruits in a semiliquid environment, and as a consequence, the larvae regularly experience episodes of hypoxia/anoxia whereas adults live in air and are likely to experience hypoxia on rare occasions. Perhaps surprisingly, larvae are much less tolerant of anoxia than adults; nearly 100% of adults survive up to 4 hours of anoxia while less than 50% of third instar larvae survive anoxic bouts greater than 90 minutes. We used spectrophotometry and H-NMR to compare the metabolite profile for anoxic larvae and adults and to test alternate hypotheses for how anoxia kills. Anoxia induced strong decreases in glycogen levels in both larvae and adults; however, both stages also showed strong increases in free glucose indicating that carbohydrate depletion is likely not a cause of death. Both larvae and adults accumulated lactate, but larvae did so at a higher rate during the first hour of anoxic exposure which suggests that high locomotion of larvae during initial exposure to hypoxia requires high rates of anaerobic metabolism that may be functionally linked to mortality. Larvae and adults utilize different metabolic pathways in anoxia—primarily in the accumulations of fumarate and acetyl-CoA precursors in anoxic larvae. Survival of a 1 hour anoxia exposure was highly repeatable but varied greatly across 178 lines from the *Drosophila* Genetics Reference Panel (DGRP)

ranging from 25-95%. Thirty-two SNPs and 23 genes were associated with the variation in survival across lines, all with P values less than 0.00001. Gene ontology enrichment analysis implicates gene classes involved in angiogenesis, cell proliferation, membrane and cytoskeletal remodeling, and fatty acid metabolism in the differential survival across lines. Several of the identified genes linked to survival across the DGRP lines have human orthologs that have been linked to multiple oxygen-mediated cancer pathways and Alzheimer's progression in humans. This research was supported by NSF IOS 1256745. .

381B

Activation of Transposable Elements Across Multiple Tissues in Aging *Drosophila*. Brian C Jones, Jason G Wood, Cheng-yi Chan, Stephen L Helfand. Molecular Biology, Cell Biology, Biochemistry, Brown University, Providence, RI.

Transposable elements (TEs) are parasitic sequences of DNA that infect host genomes and retain the capacity to mobilize. This mobilization has the potential to compromise genomic integrity by inserting into normal genes and disrupting their function. Additionally, an increase in the number of copies of a given element increases genomic repetitiveness and this can directly contribute to genomic rearrangement and double-stranded breaks. TEs have been associated with many age-associated diseases including cancer, neurodegeneration, and multiple types of cellular stressors. More recently, reactivation and mobilization of TEs has been detected in aging tissues of mice, human cell culture, and fly neurons. These findings have begun to suggest that aging itself may lead to reactivation of TEs and that this may directly contribute to the aging process. Here we present findings that further confirm the reactivation of TEs during aging in the fly. We have expanded on the findings of others to also examine TEs across multiple tissues. It is well known that different tissues in the fly are known to age at different rates. Here, we find that TE RNA levels increase with age in the head, thorax, and fat body of adult flies. Interestingly, some TEs that significantly increase with age are shared across all three tissues while some that increase with age are tissue-specific. TEs of a specific class or family are also not globally upregulated suggesting that some TEs are specifically reactivated during aging. These findings contribute new knowledge about the tissue-specific effects of aging in the context of TE reactivation and begin to hint at which TEs may contribute directly to aging in *Drosophila*.

382C

The Effects of Aging on DNA Double-Strand Break Repair. Faraz Sohail, Elisa Bienenstock, **Jeannine LaRocque**. Human Science, Georgetown University, Washington, DC.

Aging is often associated with an increase in genome instability, to which deficiencies in DNA damage repair can contribute. One hallmark of aged and premature aging cells is an increase in double-strand breaks (DSBs), a particularly deleterious type of DNA damage that if not accurately repaired, can result in cell death, mutagenesis, and cellular transformation. DSBs are repaired by multiple pathways, including homologous recombination (HR). HR is error-free, utilizing an unbroken homologous template to restore genetic information at the site of the break. It is unclear if the observed increase in DSBs in aged and premature aging cells is due to an accumulation of unrepaired DSBs over time, or an inability to accurately repair new DSBs in aged cells. We hypothesize that aging results in a decreased ability to repair DSBs via error-free HR. To test this, we have developed a novel reporter assay that allows us to create a single genomic DSB and measure repair by HR in *Drosophila melanogaster*. In this system, we can measure repair of DSBs induced at various ages, as well as measure repair of DSBs in animals defective in homologs associated with premature aging, such as Werner Syndrome. Our data demonstrates that repair by error-free HR is significantly reduced when DSBs are induced in older animals, suggesting a mechanism by which mutagenic DNA damage repair increases with age.

383A

Control of pupation timing by transcription factors Blimp-1 and FTZ-F1 in fat body during prepupal period. Haruka Nishida, Abdel-Rahman Sultan, Kazutaka Akagi, Moustafa Sarhan, Azusa Koie, Takumi Nakayama, Hitoshi Ueda. Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

Living organisms sometimes determine their developmental events at precise timing. The regulation of developmental timing is important for determination of body size and adaptation to various environmental conditions. However, molecular mechanisms for determination of developmental timing remain poorly understood. In *Drosophila melanogaster*, prepupal period, from puparium formation to pupation, is about 12 hours in standard culture condition at 25 °C. Additionally, four morphologically distinct developmental events, hatching of egg, two larval molts and eclusion of adult occur at relatively precise time too. These facts suggest that they have a timer to determine the developmental timing. We have been studying the mechanism and showed the importance of ecdysone-inducible transcription factors Blimp-1 and FTZ-F1 for the determination of prepupal period. Recent our study using temporally specific knockdown or induction of these factors revealed that these factors play important roles to determine the pupal period. Furthermore, our results indicate a critical role of fat body for the determination of the developmental timing by these factors.

384B

Evaluating toxicity of silver nanoparticles in *Drosophila melanogaster*. Ananya Sharma, Ajay Srivastava. Biology and Biotechnology Center, WKU and the Gatton Academy of Math and Science ,Western Kentucky University, Bowling Green, KY 42101.

Silver nanomaterials have found ever-increasing applications in a variety of consumer products including food, clothing, cosmetics and medicine. Current research based on cell culture studies indicates that silver nanoparticles (AgNPs) have the potential to affect human health and environment as they can enter cells and interact with DNA or cellular proteins with deleterious consequences. To better understand the effects of AgNP exposure on an intact organism we utilized the genetic model *Drosophila melanogaster*. Experiments were designed to study the distribution patterns and effects of 60, 40, 20nm size AgNPs on development and longevity

of *Drosophila melanogaster*. Our results suggest that adult flies exposed to 60 nm AgNPs result in a reduction of life span. Experiments are currently underway to quantify AgNP levels in various larval tissues using ICP-MS. Effects of 40, 20nm size AgNPs on fly life span, accumulation patterns in larvae and hemocyte population will be discussed.

385C

Identifying Novel UNC-45 Interacting Partners in *Drosophila melanogaster*. Daniel Smith[1], Carmen Carland[1], Majid Mekany[1,2], Sanford Bernstein[1]. 1) Biology Department, San Diego State University, San Diego, CA; 2) Cell and Molecular Biology Joint Doctoral Program, San Diego State University and UC San Diego, San Diego, CA.

The UNC-45 protein is a member of the UNC-45/CRO1/She4p (UCS) family that is required for normal muscle development in several model organisms. UNC-45 interacts with the chaperone Hsp90 and is hypothesized to be responsible for folding the myosin motor domain. However, additional factors that interact with UNC-45 during this process are poorly characterized. To approach this problem, we are using the *Drosophila melanogaster* model system to identify novel UNC-45 interacting proteins. We are in the process of screening chromosomal segment deficiency lines in a reduced UNC-45 background for potential UNC-45 interacting partners. Each deficiency cross is then assessed for a) synthetic lethality or b) poor flight ability due to poor muscle development. We have identified several candidate deficiency lines that exhibit poor flight ability and a single deficiency line that exhibits synthetic lethality. To date, we have investigated approximately 50% of the genome using the Exelexis deficiency kit and we plan to use the Bloomington deficiency lines to increase our coverage. We are also developing a transgenic UNC-45::GFP fusion protein that will allow us to isolate UNC-45 complexes that can be interrogated using mass spectrometry. We have shown that the protein is robustly expressed *in vitro* and appears to be correctly folded according to circular dichroism spectroscopy. We are currently working to confirm *in vitro* chaperone activity of the UNC-45::GFP fusion protein and determining whether the UNC-45::GFP fusion product is capable of rescuing UNC-45 null alleles *in vivo*. This biochemical analysis of UNC-45 interacting partners will complement our genetic screening approach and further our understanding of UNC-45's role in muscle development.

386A

The *speck* gene, discovered in 1910, is a mutation in a transcript of the *Dopamine acetyltransferase* (*Dat*) gene. Eric P. Spana, Amanda B. Abrams. Department of Biology, Duke University, Durham, NC.

The pigmentation mutation *speck* (*sp*) is a commonly used recombination marker characterized by a darkly pigmented region just beneath the wing hinge. Identified in March 1910 by Thomas Hunt Morgan, *speck* was characterized by Sturtevant as the most "workable" mutant in the rightmost region of the second chromosome and eventually localized to 2-107.0 and 60C1-2. Though the first *speck* mutation was isolated over 100 years ago, *speck* is still not associated with any transcription unit and resides as an unannotated gene model in FlyBase. In our investigation, we found that both sp^1 and sp^2 contain a 412 retrotransposon in exon 1 of the RB transcript of the *Dopamine N acetyltransferase* (*Dat*) gene. A Minos insertion in that same exon of Dat-RB also gives a strong *speck* phenotype and can be reverted to wild type. In addition, expression of Dat RNAi constructs either ubiquitously or in the dorsal portion of the wing imaginal disc phenocopies *speck*. These results lead us to conclude that *speck* is caused by a loss of Dat function. We have also identified that the wing hinge is not the only region where the *speck* phenotype manifests. We find that *speck* also presents phenotypes in a pigmented stripe at the posterior end of the pupal case, the leg joints, and overall body color, especially in stronger alleles such as sp^2 and the Minos insertion. We propose that Dat is required to acetylate dopamine as a step in the sclerotization pathway, thereby decreasing the pool available for melanin production. When Dat function is decreased, the excess dopamine enters the melanin pathway and localizes to specific regions of the body during development.

387B

Microarray analysis of *Drosophila* Cdk5/p35 kinase mutants. J. Spurrier[1,2], K. McLinden[1], E. Giniger[1]. 1) NINDS, National Institutes of Health, Bethesda, MD; 2) CMDB, Johns Hopkins University, Baltimore, MD.

Cyclin dependent kinase 5 (Cdk5) gain and loss of function have both been found to be associated with neurodegeneration (ND) in animal models. However, the mechanisms involved in Cdk5-associated ND remain unclear. Thus, mutation of the gene encoding the essential activating subunit of Cdk5, called p35, yields flies that are viable and fertile at eclosion with grossly normal brain structure, but exhibit rapid loss of motor function with age and die with a lifespan roughly half that of wild type (WT) flies. Analysis of brains from aged $p35^{-/-}$ mutants reveals two subsets of phenotypes: mutant-specific and aging-mimic. Mutant-specific phenotypes are defects that only occur in $p35^{-/-}$ flies and not in WT flies, such as proximal axonal swelling, absence/shortening of the axon initial segment, and selective tissue loss from the mushroom bodies, a region required for olfactory learning and memory. Aging-mimic phenotypes are defects that normally occur in aging wild-type (WT) flies, but are happening at an earlier chronological age in $p35^{-/-}$ mutants; these phenotypes include axonal fragmentation, increased apoptosis and necrosis, and impaired autophagy, as well as loss of motor function. Focusing on the aging-mimic phenotypes, I will investigate whether Cdk5 dysfunction leads to an acceleration of the intrinsic rate of aging. Using gene expression profiles, our lab identified a subset of genes that demonstrate consistent changes in expression as the control flies age; thus, we can use the gene expression profile as a metric for the physiological age of a fly. Our lab has shown that the profile of young 10-day old $p35^{-/-}$ mutant flies most closely resembles that of older WT flies; this holds true both when using our own control flies to generate a profile and when comparing our mutant flies to previously published microarray data from an independent lab. I will expand upon these findings by completing microarray analysis of Cdk5 gain- and loss-of-function mutants in a separate genetic background to test the robustness of the effect on aging. Additionally, I will validate the microarray results using qPCR.

388C

Activation of innate immune response induces age-related caspase activation in Or42b neuron. Ken-ichi Takeuchi[1], Takahiro Chihara[1,2], Masayuki Miura[1,2]. 1) Department of Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan; 2) CREST, Japan Science and Technology Agency (JST), Gobancho, Chiyoda-ku, Tokyo, Japan.

During normal aging or in neurodegenerative diseases, our brain functions such as cognition and memory get decline. However, the mechanisms of age-related impairments in brain function during normal aging are not well known. Here we show that age-related caspase activation and cell death of specific neuron is caused by activation of innate immune response. Recently, we found that caspase, the executor protease of apoptosis is activated in a subset of olfactory receptor neurons (ORNs), especially in Or42b neurons during normal aging (PLOS Genetics 10, e1004437, 2014). ORN is the first order neuron of *Drosophila* olfactory system, and Or42b neuron is known to be necessary and sufficient for innate attractive behavior to food-like odors. Thus, aging can affect the defined animal behavior by affecting the death of specific neurons, such as Or42b neurons. In this report, we investigate the molecular mechanism underlying the age-related caspase activation in Or42b neurons. To investigate the impact of aging on ORNs including Or42b neurons, we first performed gene expression profiling of young or aged antenna with microarray analysis. We found that expression of antimicrobial peptide (AMP) genes were significantly up-regulated in aged antenna, suggesting that innate immune response is induced in aged antenna. Consistent with this, age-related caspase activation was suppressed in mutants for innate immune response. These results indicate that caspase activation is the consequence of activated innate immune response. Our results suggest the possible link between innate immune response and age-related decline of brain functions during normal aging.

389A

Nuclear Spermatid Transition Proteins involved in chromatin condensation in *Drosophila*. Zain Alvi, Angela Klaus, Tin-Chun Chu. Department of Biological Sciences, Seton Hall University, South Orange, NJ 07079.

The current study is aimed at identifying and analyzing the conserved domains found in nuclear spermatid transition proteins in the original 12 sequenced species of *Drosophila*. These proteins facilitate the process of nuclear transformation during spermiogenesis. In *D. melanogaster* transition protein (TPL94D) aids in transitioning the histone bound sperm DNA to a protamine-like protein bound sperm DNA. We have putatively identified sequences for the TPL94D orthologs in *D. simulans*, *D. sechellia*, *D. yakuba*, *D. erecta*, *D. ananassae*, and *D. pseudoobscura* by using the reference sequences found in *D. melanogaster*. Our current work indicates that TPL94D orthologs are conserved only among the *Drosophila* species in the melanogaster subgroup and in *D. pseudoobscura* (subgenus Sophophora). There are no TPL94D orthologs conserved among the Drosophila subgenus species (*D. mojavensis*, *D. virilis*, and *D. grimshawi*). All TPL94D ortholog matches have a putative conserved DNA binding domain and an overlapping conserved high mobility group (HMG) box. RNA-Seq expression analysis on the testes revealed that the identified sequences for TPL94D orthologs are highly expressed in *D. melanogaster*, *D. simulans*, *D. yakuba*, and *D. pseudoobscura*.

390B

A function for Syncrip in the *Drosophila* male germline. Catherine Baker, Emily Taing, Margaret Fuller. Dept Developmental Biol, Stanford Univ Sch Medicine, Stanford, CA.

Translational control is critical for regulating when and where many developmentally significant proteins appear during gametogenesis, in both males and females and across species. In the *Drosophila* male germline, regulation of translation is essential for dictating the timing of protein expression over 3.5 days of spermatocyte development and 3 days of post-meiotic differentiation, as waves of transcription early in spermatocyte development produce over 1000 RNAs whose protein products are required later for meiotic entry and spermatid differentiation, respectively. Syp, the *Drosophila* homolog of the mammalian SYNCRIP/NSAP1/hnRNP Q protein involved in RNA transport, stability, splicing, and translation, is required in the male germline for execution of the meiotic divisions and post-meiotic differentiation. A meiotic arrest phenotype resulted when Syp function was knocked down by RNAi (all *syp* isoforms), or knocked out via a CRISPR-generated classical loss-of-function allele (in a testis-specific Syp isoform). An eYFP-Syp *in vivo* reporter corresponding to the testis-specific isoform is localized to the nucleus in the very earliest spermatocytes and is predominantly cytoplasmic in subsequent spermatocyte stages. Two copies of this reporter in *syp* homozygotes allow rescue of meiotic nuclear division (but not cytokinesis) and partial rescue of post-meiotic differentiation, with spermatids elongating but not to wild-type lengths. We hope to use this reporter and other tools to identify both the germline RNA targets of Syp and any Syp-interacting proteins.

391C

Characterization of the function of AAA ATPase Nmd in *Drosophila* spermatogenesis and *in vitro*. Tucker Bates, James Winkle, Karen Hales. Department of Biology, Davidson College, Davidson, NC.

The *no mitochondrial derivative* (*nmd*) gene encodes a protein (Nmd) belonging to the AAA ATPase family. The protein's amino acid sequence closely resembles those of known microtubule-severing proteins spastin, katanin, and fidgetin. In *Drosophila melanogaster* containing mutated *nmd* alleles, spermatogenesis phenotypes are characterized by incomplete cytokinesis and impaired mitochondrial locomotion and aggregation – both possible associated with failed microtubule-severing. We hypothesize that Nmd functions as a microtubule-severing protein with an important role in cell cytokinesis and mitochondrial movement and shaping. By immunostaining microtubule (+)-ends using anti-EB1 antibody in *nmd* mutant and wild type flies, microtubule length and number can be quantified. We hypothesize EB1 visualization will indicate fewer and more uniformly-sized microtubules in mutant *nmd* flies than in wild type flies. Numerous immunostaining trials have reduced sources of error and shaped a more viable protocol. Visualizing

microtubule (+)-ends through an EB1-GFP transgene in *nmd* mutants and wild type flies allows a similar understanding of *nmd*'s role in microtubule dynamics. We hypothesize EB1-GFP visualization in *nmd* mutants will reveal microtubules that are fewer in number and longer in length. Extensive crosses have verified current genotypes and near expression of the genotypes necessary for analysis. Utilizing a microtubule severing assay that employs purified Nmd protein allows visualization and quantification of microtubule severing. We hypothesize that microtubules exposed to purified Nmd in our microtubule-severing assay will be cut more frequently than those not exposed to Nmd. .

392A

The Role of *Ribbon* in the Drosophila Testis. Andrew P Droste, Diane Silva, Jennifer Jemc. Biology, Loyola University Chicago, Chicago, IL.

The gonad provides a great model for studying the regulatory mechanisms, underlying how different cell types come together during development, and the mechanisms that maintain organ structure in the adult. The gonad is composed of germ cells and somatic cells, where proper interaction of these cells is critical for proper gonad formation, as well as maintenance of a functional gonad in the adult. In previous studies, we identified *ribbon* (*rib*) as a gene that is required for gonad formation in the embryo. *rib* encodes a transcription factor of the bric-a-brac, tramtrack and broad complex (BTB) family. Mutations in BTB proteins have been linked to multiple diseases and developmental defects, including infertility. Recently, immunostaining revealed expression of *rib* in the somatic cells and germ cells in the adult testis, but not in the ovary. Although we have concluded where the gene is expressed, we are still unclear how the Rib protein functions. Through a process of clonal analysis we are removing *rib* function in germ cells and somatic cells of the male gonad to better understand the role of Rib in the adult gonad. Given that mutations in other BTB family proteins exhibit increased proliferation of spermatogonia and defects in the transition from spermatogonia to spermatocyte, we believe that Rib may have a similar function. .

393B

Dynamic expression of Suppressor of Hairy-wing [Su(Hw)] in spermatogenesis underlies a role in male fertility. Tingting Duan, Pamela Geyer. Department of Biochemistry, University of Iowa, Iowa City, IA.

Spermatogensis involves expression of nearly half of all Drosophila genes. Most of these genes are transcribed during a three-day period when primary spermatocytes prepare for meiosis. During this period, transcriptional regulation depends upon testis-specific regulators, such as the testis-specific TFIID paralogues (tTAFs) and the testis-specific meiotic arrest complex (tMAC), two complexes that counteract Polycomb group dependent repression. The role of other transcriptional regulators in spermatogenesis is less clear. We are interested in the multi-zinc finger transcription factor, Su(Hw), which functions as an insulator, activator and repressor protein. Su(Hw) is nearly ubiquitously expressed during development. One exception to this global expression occurs during spermatogenesis, wherein Su(Hw) is lost in primary spermatocytes, at the same time when the tTAFs and tMAC genes begin expression. Motivated by this observation, we studied the requirement for Su(Hw) in spermatogenesis, uncovering that loss of Su(Hw) causes age-dependent male sterility. Extending these studies, we defined phenotypes associated with aged *su(Hw)* mutant testes. We find that *su(Hw)* null testes have an expanded zone of primary spermatocytes, due to defects in spermatid elongation. Microarray analyses reveal that Su(Hw) loss alters expression of ~300 genes. We predict that 60 are direct Su(Hw) targets, as these genes carry Su(Hw) binding sites within the gene or the 2 kb upstream and downstream DNA. The majority of the mis-regulated Su(Hw) target genes are neuronal genes that become activated upon Su(Hw) loss. Based on these data, we conclude that Su(Hw) is a transcriptional repressor in the testes. We are currently investigating how gene expression changes are linked to defective spermatid differentiation.

394C

Phospholipid-derived signaling molecules in *Drosophila* spermatogenesis. Yosef Frenkel, Eli Miller, Josefa Steinhauer. Department of Biology, Yeshiva University, New York, NY.

In many well-studied cell communication pathways, extracellular proteins act as messengers between cells, but certain lipids also can act as communication mediators. Fatty acids, which are precursors for potent lipid signaling molecules, are stored in membrane phospholipids and are released by phospholipase A_2 (PLA_2). Lysophospholipid acyltransferases (ATs) oppose PLA_2 activity, re-esterifying fatty acids into phospholipids, in a biochemical pathway known as the Lands Cycle. Following release from phospholipids, fatty acids are metabolized into signaling lipids such as prostaglandins, which play key roles in mammalian immunity and fertility. We are investigating the function of the Lands Cycle and phospholipid-derived signals in *Drosophila*. BLAST analysis reveals ten predicted PLA_2 genes in the*Drosophila* genome. RNAi against each PLA_2 gene individually does not cause lethality or obvious developmental defects. We are testing for redundancy amongst the PLA_2s. We have shown that*Drosophila* Lands Cycle ATs Oys and Nes are required for spermatid individualization, suggesting an evolutionarily conserved role for this pathway in male fertility. RT-PCR shows that five PLA_2 genes are expressed in the testis. We are using RNAi, overexpression, and in situ hybridization to investigate PLA_2 function there.

Furthermore, we have found that mutants for the *Drosophila* cyclooxygenase Pxt, which creates prostaglandins from fatty acids, also show spermatid individualization defects. Our results suggest that specific lipid signals, whose abundance is regulated by the Lands Cycle, are important regulators of spermatogenesis.

395A

Characterization of age related effects on spermatogenesis in the DGRP. Michelle Giedt, Douglas Harrison. Biology, University of Kentucky, Lexington, KY.

The reproductive lifespan of an organism is dependent upon the contribution of a number of genetic and environmental factors that

regulate homeostatic and developmental processes. Loss of or changes in tissue homeostasis and the subsequent differentiation of stem cells into gametes impact fertility and fecundity. While the genetics of lifespan have been well studied, genes driving reproductive senescence are less well characterized. Reproductive tissues have an easily followed stem cell pool, high rate of developmental activity, and are dispensable for organism viability making them a tractable model tissue for aging studies. Using the DGRP, a set of >200 publicly available wild derived inbred lines, we are characterizing genetic and cellular aspects of male reproductive aging. Given the high density of variants within the DGRP, there is a high probability that loci associated with reproductive aging can be identified. Currently we are conducting reproductive lifespan assays on each line to determine the average age at infertility. Results thus far show shortened reproductive lifespans compared with wild-type, most likely due to inbreeding depression and variation in age at reproductive senescence between DGRP lines. To address whether age related changes at the cellular level are predictable or vary between individuals, we aged and dissected males from several DGRP lines encompassing a range of reproductive lifespans. Using germline and proliferation markers, we observe between line differences in the pattern of aging. DGRP_358 becomes infertile earlier than wild-type and exhibits defects in germline proliferation, differentiation, and sperm maturation with age. Similar changes are observed in wild type males at later ages, suggesting accelerated senescence in DGRP_358 males. Sequence data from this line will be analyzed to identify variants responsible for obvious protein changes to identify potential candidate loci resulting in the observed senescence phenotype. These results suggest that there is a genetic contribution to reproductive lifespan and, that while variability exists between individuals, there are common morphological changes that occur during reproductive aging.

396B

Characterization of AAA ATPase proteins Nmd and CG4701 through an analysis of mitochondrial dynamics, cytokinesis, and microtubule organizing centers during *Drosophila melanogaster* spermatogenesis. Devon Harris, Bethany Wagner, Sarah Pyfrom, Jessica Gerard, Melissa Lorenzo, James Winkle, Lindsay Regruto, Karen Hales. Davidson College Department of Biology, Davidson College, Davidson, NC.

Nmd and CG4701 have putative microtubule severing functions based on their amino acid similarities to proteins with known microtubule interacting functions, such as spastin and katanin. Mitochondrial shaping defects during *Drosophila melanogaster* spermatogenesis in flies with mutations in either gene suggest a connection between mitochondrial shaping and microtubule dynamics. Male flies with a mutation in the untranslated region of *nmd* lack a mitochondrial derivative, called the Nebenkern, due to an aggregation and fusion defect. Another mutant allele of *nmd* with a mutation in the ATP binding domain causes large Nebenkern with four nuclei per cell, indicative of cytokinesis failure. Mutations in *CG4701*, a testis specific paralog of *nmd*, result in a large, vacuolated Nebenkern with multiple nuclei in each cell. Nmd-GFP and CG4701-RFP localize to mitochondria and nmd-GFP additionally localizes to microtubule organizing centers. Nmd-GFP rescues *nmd* mutants. Rescue experiments with CG4701-RFP show a partial rescue of the *CG4701* mutant phenotype. Cytokinesis failure could result from malformed contractile rings if disrupted microtubule dynamics prevent signaling for a contractile ring to form at the correct time and location, resulting in a diffuse rather than constricted contractile ring. Two fluorescently labeled proteins that are components of the contractile rings are used to visualize these structures. GFP-Anillin localizes to the cleavage furrow and is present in ring canals of wild type and mutant spermatids, suggesting that *nmd* and *CG4701* mutants do have constricted contractile rings. The defect could be later in the process after Anillin functions, which will be tested with Septin2-GFP localization in mutants. The dual localization phenotype of Nmd and previously observed multiple centrosomes in *CG4701* mutants indicate that microtubule organizing centers could be disrupted in *nmd* and *CG4701* mutants, which will be visualized using Unc-GFP.

397C

Snail is required for maintenance of male germline stem cells. Gary Hime[1], Aviv Gafni[1], Arjun Chahal[1], Agnes Gany[1], Franca Casagranda[1], Nicole Siddall[1], Kate Loveland[2], Helen Abud[2]. 1) Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia; 2) Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia.

The Snail family of transcriptional factors have long been implicated in epithelial to mesenchymal transitions but have also have roles in regulation of cell proliferation, adhesion, survival and stem cell identity. We have analysed the role of this family in male germline stem cells (GSCs) and identified a requirement for Snail function to maintain GSCs within the niche. *sna* mutant GSC clones are not maintained, in contrast to *esg* clones. *sna* mutant GSCs appear to lose adhesion to the hub, a group of somatic cells that form part of the GSC niche. Mutant GSCs do not apoptose and are capable of differentiating into spermatocytes. *sna* mRNA is present at very low levels in the testis but can be detected by droplet digital PCR. Expansion of the stem cell pool in the testis via ectopic expression of *unpaired* results in a greater than 10 fold increase in *sna* mRNA but does not permit detection of Snail protein via immunofluorescence. In contrast, Snail protein can be detected in GSCs if ectopically expressed using nosGal4 and this causes a significant increase in GSC numbers and increased GSC mitotic activity. We are currently conducting targeted DamID to identify Snail targets in the germline.

398A

A Visual Screen for Centriolar Mutants. S. Hynek, T. Janowicz, S. Baghdy, K. Arum, A. Marcano, M.S. Choi, N. Scheanwald, T. Avidor-Reiss. Biological Sciences, University of Toledo, Toledo, OH.

Centrosomes are conserved organelles that function as microtubule organizing centers during cell division and fertilization. In addition, centrioles function to template the cilia. Defects in the centrosome result in a variety of diseases, including blindness, infertility, and cancer, and yet our knowledge of how centrosomes form and function is incomplete. To gain insight into the biology of

the centrosome, we study it in *Drosophila melanogaster*. Using *Drosophila* as a model system for centrosome defects is advantageous because centrosome mutants can develop into adults. In *Drosophila*, centrioles are required to sense the environment, for meiotic division, and sperm motility. During sperm formation (spermatogenesis), centrosomes in the testes undergo many changes including duplication, elongation, separation, and reduction. Until now, *Drosophila* centrosomes were studied systematically by reverse genetics using known genes from other organisms, or by using genomic or molecular approaches. Centrosomal mutants have also been identified in forward genetic screens for mechanosensation and sperm function defects. Here, we have performed, for the first time, a visual forward genetic screen of *Drosophila* testes directed to identify centrosomal mutants. Mutations were induced by using ethyl methanesulfonate. Testes were chemically fixed, then analyzed using fluorescence microscopy. Centriolar mutants were identified using the centriole marker, Ana1-GFP, and other mutations with nuclear staining using Hoechst. Currently, 1353 mutants have been screened; 23 centriolar mutants were isolated and 412 mutants were isolated with phenotypes such as abnormal nuclear and testes morphology, and lacking mature sperm. Many of the centriole mutations affect the length of Ana1 labeling, suggesting that these mutations affect centriole length. Some of the centriole length mutants fail to complement Poc1, Bld10, and Cep290, potentially representing new alleles of these centriolar proteins. Many of the new centriolar mutants do not exhibit robust sensory or meiotic phenotypes, rendering this visual screen a novel and effective way to identify new centrosome mutations.

399B

Overexpression of *erpL22* leads to increased proliferation, decreased fertility, and a shortened lifespan. Catherine Mageeney, Alex Chen, Jennifer Colquhoun, Michael Kearse, Vassie Ware. Biological Sciences, Lehigh University, Bethlehem, PA.

Drosophila ribosomal protein (RP) eRpL22 is ubiquitously expressed and essential. Previously, we have reported L22 is differentially SUMOylated in testis compared to bodies, and localizes to the nucleoplasm of meiotic spermatocytes. SUMOylated L22 function is unknown. Computational analysis of L22 predicts SUMO sites within a fly-specific N-terminal H1-like domain. Here we have explored effects of overexpression of *L22* and an N-terminal truncation of *L22*. Ubiquitous overexpression of *L22* using an *actin*-Gal4 driver revealed a testis mass, minimal sperm production, decreased fertility, and a shortened lifespan. Western blot analysis shows that newly synthesized L22 is present in both testes and bodies of adults, but is not SUMOylated. What domain(s) in L22 confers the observed phenotypes is unknown. To address whether or not the N-terminal domain plays a role in overexpression phenotypes, we overexpressed N-terminally truncated *L22* using the *actin*-Gal4 driver. In this case, truncated L22 was detectable in bodies but not in testes, and *L22* overexpression phenotypes were absent. The mechanism accounting for the lack of N-terminally truncated L22 in testes is unknown. Whether or not SUMOylation has a role in testis-specific stabilization of L22 remains to be explored. Two additional overexpression strategies were used to investigate the apparent increase in cellular proliferation seen *in vivo*. Cell-type specific drivers were used for *L22* overexpression to determine which cell/tissue types might contribute to the testicular mass. Using cell type-specific drivers for overexpression of *L22* in either germ cells, cyst cells (cc), accessory gland (ag)/cc, or ag/seminal vesicles, *actin* overexpression phenotypes were only phenocopied with an accessory gland/cyst cell driver. Therefore, *L22*overexpression within cyst cells may contribute to testicular mass production. Further, *L22* overexpression within S2 cells caused increased cell counts, suggesting an increase in cell proliferation or a decrease in cell death. The majority of overexpressed L22 remains nuclear, implicating L22 effects on nuclear events. Collectively, these results implicate eRpL22 in extraribosomal roles that may include cellular proliferation pathways.

400C

Notch Signaling is Active in Stem Cell Lineages of Testes. C. Ng, C. Schulz. Department of Cellular Biology, University of Georgia, Athens, Athens, GA.

Spermatogenesis in *Drosophila melanogaster* testes is predicated on the proper interaction between germline cells and their microenvironment somatic cyst cells. Germline stem cells (GSCs) and somatic cyst stem cells (CySCs) are located at the apical tip of the testis where they go through asymmetric division to produce new stem cells and daughter cells. The daughter cells for the GSCs called gonialblasts are fully enclosed by the CySCs daughter cells, the cyst cells. The gonialblasts undergo transit amplifying divisions to produce clusters of precursor cells called spermatogonia that eventually develop into spermatids to produce fertile sperm. Throughout this process, the surrounding cyst cells grow in size and co-differentiate with the enclosed germ cells. The Notch signaling pathway relies on the membrane-bound ligand expressed by the signal-sending cell binding to the Notch transmembrane surface receptor on the signal-receiving cell. This signaling event leads to activation of Notch target genes in the receptor-expressing cell. Notch signaling appears to play a role in the early stages of spermatogenesis. Antibodies against Notch signaling components localize to the apical tip of the testes within the cellular membranes of early stage cells. Knockdown of Delta in the germline leads to germline loss while hyperactivation of Notch signaling in cyst cells results in failure of cyst cells and germline to differentiate properly. These cyst cells express both early and late stage molecular markers simultaneously and the germline fail to develop into spermatids. We hypothesize that Delta signals from the germline to the Notch receptor on the encompassing somatic cyst cells to prevent germline and cyst differentiation. We propose that activation of Notch in cyst cells prevents their premature differentiation and subsequently differentiation of the germline.

401A

Differential gene expression associated with X-linked sex-ratio meiotic drive in *Drosophila affinis*. Robert Unckless, Andrew Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Sex-ratio meiotic drive occurs when the heterogametic sex harbors a sex chromosome (usually the X) that is able to bias its own transmission and distort the offspring sex-ratio in its favor. Several sex-ratio (SR) systems are known in Drosophila, other Dipterans,

mammals and plants. In an attempt to understand the genetic causes and consequences of sex-ratio meiotic drive in *Drosophila affinis*, we sequenced RNA from testis and carcasses of sex-ratio and standard (ST) males that shared the same autosomes and Y chromosome. We assembled the transcriptome and quantified expression levels for each transcript. Several transcripts were differentially expressed (using a false discovery rate of 0.001) between SR and ST male testis. As expected, most differentially expressed transcripts were X-linked. Using a previously determined classification scheme from *D. melanogaster*, we found that differentially expressed genes were more likely to be genes expressed post-meiotically than those expressed during mitosis or meiosis. In addition to measuring expression in males with a susceptible Y chromosome, we also measured expression in males with a resistant chromosome. Several genes that were differentially expressed between sex-ratio and standard males with a susceptible Y chromosome were not differentially expressed in males with a resistant Y chromosome providing more clues to the processes involved in the sex-ratio phenotype. .

402B

Three RNA Binding Proteins Form a Complex to Promote Differentiation of Germline Stem Cell Lineage in *Drosophila*. Di Chen[1,2], **Chan Wu**[1,2], Shaowei Zhao[1,2], Qing Geng[1,2], Yu Gao[1,2], Xin Li[1,2], Yang Zhang[1,2], Zhaohui Wang[1,2]. 1) State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; 2) The University of Chinese Academy of Sciences. The first two authors contributed equally to this work. *Correspondence: zhwang@genetics.ac.cn

In regenerative tissues, one of the strategies to protect stem cells from genetic aberrations, potentially caused by frequent cell division, is to transiently expand the stem cell daughters before further differentiation. However, failure to exit the transit amplification may lead to overgrowth, and the molecular mechanism governing this regulation remains vague. In a *Drosophila* mutagenesis screen for factors involved in the regulation of germline stem cell (GSC) lineage, we isolated a mutation in the gene *CG32364*, which encodes a putative RNA-binding protein (RBP) and is designated as *tumorous testis* (*tut*). In *tut* mutant, spermatogonia fail to differentiate and over-amplify, a phenotype similar to that in *mei-P26* mutant. Mei-P26 is a TRIM-NHL tumor suppressor homolog required for the differentiation of GSC lineage. We found that Tut binds preferentially a long isoform of *mei-P26* 3'UTR and is essential for the translational repression of *mei-P26* reporter. Bam and Bgcn are both RBPs which have also been shown to repress *mei-P26* expression. Our genetic analyses indicate that *tut*, *bam*, or *bgcn* is required to repress *mei-P26* and to promote the differentiation of GSCs. Biochemically, we demonstrate that Tut, Bam, and Bgcn can form a physical complex in which Bam holds Tut on its N-terminus and Bgcn on its C-terminus. Our *in vivo* and *in vitro* evidence illustrate that Tut acts with Bam, Bgcn to accurately coordinate proliferation and differentiation in *Drosophila* germline stem cell lineage.

403C

Disrupting Laminin function reveals an unexpected role for an ovarian muscle tissue in shaping the *Drosophila* egg. Darcy Andersen, Sally Horne-Badovinac. Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

The *Drosophila* egg chamber is an organ-like structure that lengthens along its anterior-posterior (AP) axis as it develops to create the elliptical shape of the egg. The follicle cell epithelium and its adjacent basement membrane (BM) are key players in this process. Fibril-like structures in this BM are oriented perpendicular to the egg chamber's AP axis. This structured matrix is thought to function as part of a "molecular corset" that resists the expansive growth of the germ cells, thus biasing the direction of egg chamber growth. Within the ovary, egg chambers are also surrounded by a tubular sheath of muscle that pushes the maturing eggs toward the oviduct. Interestingly, the contractile muscle fibers are oriented in the same direction as the molecular corset; however, the effect the muscle has on egg chamber elongation is unknown. To further elucidate the mechanisms governing this process, we are investigating the requirement for Laminin, a major component of the BM. Flies have two Laminin isoforms: LanA and LanW. Using tissue-specific RNAi expression, we show that, whereas LanA is required in both the follicle cells and the muscle sheath for egg chamber elongation, LanW is required only in the muscle sheath. Loss of Laminin or its transmembrane receptor Dystroglycan from the muscle initially induces hyper-contraction, which then leads to muscle necrosis and a hypo-contractile state through prolonged RNAi expression. Interestingly, this progressive muscle degeneration has opposing effects on egg shape, leading first to hyper-elongation and then to rounding. The hyper-elongated egg appears to result from increased muscle compression augmenting the function of the molecular corset. In contrast, the muscle sheath's normal function in shaping the egg is through stimulating germ cell growth. Our data suggest that muscle contraction promotes vitellogenesis, and that the resulting oocyte expansion specifically increases the egg's length but not its width. These data highlight the complex and critical roles that surrounding tissues can play in organ morphogenesis.

404A

Evidence that the planar polarized localization of Fat2 in *Drosophila* follicle cells depends on microtubule-based transport. F. Aurich, C. Dahmann. Technische Universität Dresden, Dresden, Germany.

Planar cell polarity is an important feature of developing epithelial tissues. In the *Drosophila* ovarian follicle epithelium, planar cell polarity is for example evident by the alignment of actin filaments and microtubules at the basal plane of the tissue perpendicular to the anteroposterior axis of the egg chamber. In addition, proteins of the extracellular matrix form fibrils that align parallel to the actin filaments and microtubules. This alignment of extracellular fibrils is driven by a rotation of the egg chamber relative to its extracellular matrix. The aligned extracellular matrix fibrils are thought to provide a molecular corset during egg chamber growth that facilitates egg chamber elongation. The atypical cadherin Fat2 is required for egg chamber elongation, egg chamber rotation, and the proper alignment of microtubules and actin filaments. Fat2 protein localizes to cell junctions at the basal side of follicle cells and is enriched at cell junctions oriented parallel to the long axis of the egg chamber. The planar polarized localization of Fat2 depends on intact microtubules. However, the detailed molecular mechanism by which the localization of Fat2 is polarized remains unclear. Here we use

live imaging of a GFP-tagged form of Fat2 to analyze the dynamics of Fat2 protein in follicle cells. We observe intracellular Fat2-GFP particles that preferentially move parallel to the orientation of microtubules and that get in close proximity to the plasma membrane. The velocity of the Fat2-GFP particles is consistent with motor-dependent transport processes. Depolymerization of microtubules, but not of actin filaments, stalls Fat2-GFP particle movement. These experiments are consistent with the view that a directed microtubule-dependent transport of Fat2 is involved in the polarized localization of Fat2. .

405B

Evidence the dot chromosomes help organize chromosome movements during female meiotic prometaphase I in *Drosophila melanogaster*.. William Gilliland, Eileen Colwell, David Osiecki. Department of Biological Sciences, DePaul University, Chicago, IL.

A common genomic feature of many Drosophilid species is a small dot chromosome. In *Drosophila melanogaster* the dot chromosome is chromosome *4;* this chromosome is highly heterochromatic, and normally never participates in meiotic crossing over. While crossing over is usually required to lock homologous chromosomes together until anaphase I, the *4s* are connected through recently discovered tethers made from the heterochromatin, which enable nonexchange nonexchange chromosomes to ensure coorientation when the *4s* move out on the spindle during prometaphase I, followed by congression to a compact mass at metaphase I arrest. We have been investigating the effects of a number of different aberrant chromosome configurations on prometaphase I chromosome movement during congression in *D. melanogaster* female meiosis. These aberrations include compound chromosomes, where the genes normally found on two chromosomes have been rearranged so they are connected to a single centromere. Examination of meiotic spindles in females with these aberrant configurations reveals that changes to chromosome *4* can cause significant changes in chromosome movement and positioning, and that prometaphase chromosome movements are reduced in females without two free chromosomes *4*. We also provide cytological evidence that the size of the heterochromatic sequence blocks can alter the way chromosomes move during prometaphase I. Together, these results suggest that the role of the enigmatic dot chromosome may be to help organize the movement of all chromosomes during prometaphase I.

406C

Blm and mei-MCM Complex Coordinately Regulate Crossover Control in *Drosophila*. Talia Hatkevich, Kathryn Kohl, Susan McMahan, Jeff Sekelsky. University of North Carolina Chapel Hill, Chapel Hill, NC.

Meiotic crossover (CO) formation is essential to prevent improper homologous chromosome segregation, the leading cause of genetic birth defects such as Trisomy 21 and spontaneous miscarriages. Due to the importance of COs, CO formation is highly regulated. The encompassing regulation of CO formation is referred to as CO control, and the mechanisms that implement genome-wide CO control are poorly understood. Through recent studies with the anti-CO helicase Blm and the pro-CO structure mei-MCM complex, we have gained insight into how crossovers are regulated in *Drosophila*. In *Drosophila*, a typical CO distribution shows a mild distal inhibitory effect and a strong centromeric inhibitory effect, with most COs frequently placed in the middle of the arm. Our lab finds that the placement of COs is disrupted in *Blm* and *mei-MCM* mutants; in *Blm* single and *Blm mei-MCM* double mutants, the wildtype distribution of COs is completely lost, indicating that CO placement is random. Unexpectedly, the *mei-MCM* mutant shows a disruption only in the proximal region of the arm, indicating that the *mei-MCM* complex is partially responsible for the strong CO inhibition effect at the centromere. CO control systems assure at least one crossover on each chromosome, and in *Drosophila*, an average of 2.2 COs are found on each chromosome. Our studies show that CO guarantee is lost in *Blm mei-MCM* single and double mutants. The effect of Blm and the mei-MCM complex on interference, the phenomenon that prevents closely spaced COs, is currently being investigated. Taken together, our results show that BLM and the mei-MCM complex disrupt CO control. The mechanism to which these proteins play a role is elusive, though. To provide insight into the mechanism of CO control, we are investigating the separation-of-function mutant of REC, a member of the mei-MCM complex. Thus far, our studies show that a mutant Walker B motif in REC reduces the CO frequency to levels comparable to *rec* mutant. Results from our current studies will provide additional knowledge to help unravel the mechanisms behind CO control. .

407A

A tunable, secretion-based mechanism for basement membrane remodeling during egg chamber elongation. Adam Isabella[1,2], Sally Horne-Badovinac[1,2]. 1) Department of Molecular Genetics & Cell Biology, University of Chicago, Chicago, IL; 2) Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL.

During development, basement membranes (BMs) are continually remodeled to accommodate changes in tissue size and shape. Importantly, BM remodeling can also play an instructive role in directing organ morphogenesis. Very little is known, however, about the molecular and cellular mechanisms that control BM dynamics in developing tissues. We are using the *Drosophila* egg chamber as a highly tractable system to study BM remodeling during organ morphogenesis *in vivo*. Though initially spherical, egg chambers elongate dramatically as they grow. This morphogenesis depends on an external BM that is secreted and remodeled by underlying follicular epithelial cells. Egg chamber elongation correlates with the appearance of fiber-like Col IV aggregates within the BM that are polarized perpendicular to the elongation axis. Formation of these fibers requires rotation of the egg chamber within the BM in the direction of polarization. Col IV fibers are proposed to drive elongation by constraining growth along the polarization axis. By imaging the process of fiber formation live in cultured egg chambers, we found that Col IV fibers polymerize from newly secreted protein prior to BM incorporation. Fiber formation correlates with accumulation of extracellular Col IV between lateral cell membranes. Additionally, the small GTPase Rab10, which regulates polarized BM secretion, accumulates on lateral membranes during this period. These observations suggest that Col IV secretion out the lateral membrane promotes fiber aggregation. We confirm this concept by showing that

manipulating the activity of the Rab10 pathway alters the timing and extent of lateral Col IV secretion and fiber formation. These data reveal a tunable, secretion-based mechanism to regulate BM structure during tissue morphogenesis.

408B

The Ecdysone and JAK/STAT pathways synergize to suppress Notch-induced Broad for proper morphogenetic movement in the follicular epithelium. Dongyu Jia, Jamal Bryant, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

Previously, we demonstrated that the early uniform pattern of Broad (Br), a small group of zinc-finger transcription factors resulting from alternative splicing, in the follicular epithelium is established by Notch signaling at stage 6, through the direct binding of the Notch nuclear effector, Suppressor of Hairless, to the *br* early enhancer (*brE*) region during *Drosophila* oogenesis (Jia et al., 2014). Here, we report that regulation of *br* expression is an important step for follicle cell morphogenesis, including stretched-cell stretching and border cell migration. We find the uniform pattern of Br in the follicular epithelium is gradually lost in the anterior follicle cells (stretched cells and border cells) from stage 8 to 10a. This downregulation of Br appears functionally significant, as misexpression of Br-Z1 in these two groups of cells blocks stretched-cell stretching and border cell migration. We also find Ecdysone and JAK/STAT signaling mediates the downregulation of Br. The two signaling activities are upregulated in the anterior cells from stage 8, complementary to decreased Br expression, and both are required for proper stretched-cell stretching and border cell migration. The Ecdysone pathway has been known to regulate the timing of border cell movement. Our model suggests a pulse of Ecdysone activity at stages 8/9 leads to downregulation of Br, which in turn de-represses Ecdysone activity, leading to a positive feedback loop to continuously upregulate Ecdysone activity and downregulate Br. Meanwhile, downregulated Br also de-represses JAK/STAT activity, which further suppresses Br. In summary, Ecdysone and JAK/STAT synergize to suppress Br in order to de-repress themselves for proper anterior cell movement. Our study reveals that Br interacts with the Notch, Ecdysone and JAK/STAT pathways, serving as an important spatiotemporal cue for proper cell differentiation and movement. .

409C

Characterizing a Role for the Misshapen Kinase in Growth of the Germline Ring Canals in the Developing Egg Chamber. Ashley Kline, Lindsay Lewellyn. Department of Biological Sciences, Butler University, Indianapolis, IN.

Each fly egg develops from a multicellular organ-like structure called an egg chamber; the process of oogenesis can be divided into 14 morphologically distinct stages. The developing egg chamber is composed of a central cluster of 16 germ cells (1 oocyte and 15 nurse cells) that is surrounded by a layer of somatic epithelial cells, the follicle cells. The 15 nurse cells are connected to each other and to the oocyte through large intercellular bridges called ring canals. The ring canals are f-actin-rich structures that are formed through incomplete cytokinesis during formation of the germ cell cluster within the germarium. Over the course of egg chamber development, the ring canals are stabilized by the recruitment of additional f-actin and actin-binding proteins, and they undergo a significant expansion to reach a final diameter of ~10 µm. During stage 11 of oogenesis, the nurse cells rapidly transfer their cytoplasmic contents to the oocyte, leading to a doubling of its volume in ~30 minutes. Because the oocyte remains transcriptionally inactive through most of oogenesis, the integrity of the ring canals is essential to allow the nurse cells to deliver mRNA, protein, and organelles to the developing oocyte. Although a number of structural and regulatory components of the ring canal have been identified, there is still much to be learned about ring canals formation, stabilization, and expansion. We have found a novel role for the Ste20 kinase Misshapen (Msn) in growth of the ring canals. A Msn-YFP protein trap localizes to the ring canals. Depletion of Msn by RNAi does not disrupt ring canal formation or the recruitment of other ring canal components, such as Hts-RC, Kelch, and Cheerio. However, beginning at stage 5 of development, we see a significant defect in ring canal expansion, often leading to ring canal collapse. Further genetic experiments will be necessary in order to identify the molecular mechanism and timing underlying the role for Msn in ring canal stabilization and/or growth.

410A

Diversification and redundancy of Drosophila septins *Sep2* and *Sep5*. Ryan O'Neill, Denise Clark. Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada.

Septins are a family of proteins that form hetero-oligomeric complexes. They function at the interfaces between the cytoskeleton and membranes, taking part in many processes, including cytokinesis. *Drosophila melanogaster* has 5 septin genes: *Sep1*, *Sep2*, *Pnut*, *Sep4*, and *Sep5*. Septins are grouped based on phylogeny and interchangeability within complexes. Mammalian septin complexes contain members of SEPT2, SEPT6, and SEPT7 subgroups, arranged 2-6-7-7-6-2. *Sep2* and *Sep5* are the only SEPT6 subgroup septins in *D. melanogaster*, so they are likely interchangeable within complexes. *Sep5* arose via retrotransposition of *Sep2* in the ancestor of the subgenus *Sophophora*. Multiple sequence alignment indicates functional divergence of *Sep2* and *Sep5*, as ~14% of amino acids were substituted between paralogs and are highly conserved across orthologs. Our work assesses redundancy and diversification of *Sep2* and *Sep5*. We generated null mutants for *Sep2* and *Sep5*. *Sep2* mutants are semisterile. *Sep2* mutant egg chambers can have more or less than the wild-type 15 nurse cells; some have defects in germline cystoblast divisions, indicated by abnormal fusome morphology, and others have defects in follicle cell encapsulation resulting in fused egg chambers. *Sep2* cDNA or *Sep2-GFP* transgenes rescue this *Sep2* mutant egg chamber phenotype, but a *Sep5* cDNA only partially rescues it, suggesting that Sep2 and Sep5 have functionally diverged. *Sep5* mutants appear to be wild type. However, *Sep2 Sep5* double mutants lack imaginal discs and have early pupal lethality, showing that *Sep2*and *Sep5* have a redundant function in imaginal cell proliferation. We characterized Sep2 localization in oogenesis using Sep2-GFP. Sep2-GFP localizes to the contractile ring during cystoblast divisions, and remains as part of the outer rim

of the ring canals through oogenesis. Sep2-GFP also localizes to follicle cell ring canals. Similarly, Sep5-GFP localizes to ring canals, suggesting that Sep2 and Sep5 have a conserved mechanism for localization. However, since *Sep5* only partially rescues the *Sep2* phenotype in oogenesis, it appears that some other aspect of function has diverged between them.

411B

Diversity of epithelial morphogenesis among drosophilid eggshells. Miriam Osterfield[1], Trudi Schüpbach[1], Eric Wieschaus[1,2], Stanislav Shvartsman[1]. 1) Lewis-Sigler Inst, Princeton Univ, Princeton, NJ; 2) Howard Hughes Medical Institute (HHMI), USA.

The eggshells of drosophilid species provide a powerful system for studying the origins of morphological diversity. In particular, the dorsal appendages, or respiratory filaments, of these eggshells display a remarkable interspecies variation in number and shape. To analyze the morphogenesis of dorsal appendage formation in multiple species, we developed an improved 3D image reconstruction approach. This approach revealed considerable interspecies variation in the cell shape changes and neighbor exchanges underlying appendage formation. Most strikingly, although the appendage floor in *D. melanogaster* is formed through spatially ordered neighbor exchanges, the same structure in *Scaptodrosophila pattersoni* is instead formed through extreme changes in cell shape. Others species, such as *Drosophila funebris,* display a combination of both cellular mechanisms. These results suggest that species deploy different combinations of apically- and basally-driven mechanisms to convert a two-dimensional primordium into a three-dimensional structure. Our results also imply a self-organized, post-transcriptional origin for the variability of appendage number in *Scaptodrosophila*. Our current work is focused on characterizing the molecules underpinning this process.

412C

***small ovaries* (*sov*) is required for establishment and maintenance of the *Drosophila melanogaster* ovary. Cale Whitworth**[1], Kevin Cook[2], Brian Oliver[1]. 1) National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA; 2) Bloomington Drosophila Stock Center, Dept. of Biology, Indiana University, Bloomington, IN, USA.

Identification of genes required for sex-specific development of bipotential organs are an important step in understanding how the sexual fate of an organ is specified and maintained. Previous studies have shown that one such gene, *small ovaries* (*sov*), functions in the ovarian soma, but not the germline, and is required for proper ovary development. Hypomorphic alleles of *sov* result in female-specific infertility arising due to impaired *sov* function at two stages in ovarian development. First, sov is required to establish wild type numbers of germaria as mutations result in fewer germaria per ovary in newly eclosed females. Second, *sov* is required to maintain ovary function in adult flies. In *sov* mutants, germline stem cells (GSCs) are inappropriately surrounded by follicle cells that physically separate GSCs from their stem cell niche. This results in *in situ* differentiation of GSCs followed by caspase-mediated apoptosis. We have mapped *sov* alleles to a region containing a protein-coding gene, CG14438, and a non-coding RNA, CR43496. RNA expression profiling of *sov* mutants reveals point mutations in CG14438 suggesting that its function is affected by *sov* mutations. Further, an antibody raised against CG14438 reveals expression in the soma and germline of the ovary with no detectable expression in the testis. .

413A

Imaginal disc growth factors regulate dorsal-appendage tube morphogenesis. Sandra Zimmerman, Celeste Berg. Department of Genome Sciences, University of Washington, Seattle, WA.

Morphogenetic cues from distinctly different cell-types give rise to respiratory organs called dorsal appendages (DAs) on the eggshell of *D. melanogaster*. During oogenesis, two patches of somatic follicle cells that lie dorsal to the oocyte form the DAs by reorganizing into tubes and crawling over the adjacent, squamous, "stretch" follicle cells. The Sox transcription factor, Bullwinkle (BWK), functions in the germline nurse cells to regulate DA formation; it acts through the tyrosine kinases SHARK and SRC42A in the overlying stretch cells. Mutations in *bwk* or *shark* lead to DA-cell-adhesion defects, aberrant cell migration, open tubes, and moose-antler-like DAs. Similar errors in neural tube formation cause birth defects such as spina bifida. Other components of the pathway and the signals between the cell-types are unknown. To discover how this pathway regulates DA formation via the stretch cells, we adapted a magnetic-bead cell-separation protocol for a novel application: mass spectrometry. We purified stretch cells from wild-type vs. *bwk* egg chambers, compared relative protein expression, and identified differentially-expressed proteins, including cytoskeletal regulators and signaling molecules. Intriguingly, members of a novel family of growth factors, Imaginal disc growth factors (IDGFs), which have vertebrate orthologs, were upregulated in stretch cells from *bwk* egg chambers. Stretch-cell-specific RNAi and mRNA localization analyses reveal unusual localization patterns for some IDGF mRNAs and suggest that precise levels of IDGFs non-autonomously regulate DA formation. The receptor is unknown but published cell-culture studies suggest the insulin receptor is involved. Other questions remain: Do the six IDGFs function together? Are they necessary and sufficient to guide tubes and ensure tube closure? We are using RNAi, mutant alleles, misexpression constructs, and mosaic analysis to determine the role of these newly identified factors in DA morphogenesis. Our results demonstrate the power of using magnetic-bead cell separation and mass spectrometry to reveal differential protein expression and identify morphogenetic factors in a small population of cells that non-autonomously regulate tube formation.

414B

Studying the *cis*- and *trans*- regulation of *Sex lethal* in the germline. Raghav Goyal, Pradeep Bhaskar, Mark Van Doren. Biology Department, Johns Hopkins University, Baltimore, MD.

In *Drosophila*, sex-determination is under the control of the "switch" gene *Sex lethal* (*Sxl*). While in some species the sex of the soma is sufficient to determine the sex of the germline via inductive signaling, sex-determination in the *Drosophila* germline also occurs cell-autonomously via intrinsic signaling as dictated by their chromosomal constitution. Interestingly, when *Sxl* is expressed in XY GCs, these

GCs are able to produce eggs upon transplantation into a female somatic gonad, demonstrating that even in the germline, *Sxl* is the "switch" and is sufficient to activate female identity. In both the germline and soma the presence of two X chromosomes leads to *Sxl* expression. However, its control is different in the germline than in the soma at both, *cis*- and *trans*- regulatory levels, and we are studying how this is regulated in the germline. The X chromosome genes known to be important for activating *Sxl* in the soma are not thought to be required in the germline. Further, the segments responsible for germline *Sxl* promoter activity have not been identified. We will utilize the X chromosome duplication collection to identify regions of the X chromosome important for *Sxl* activation in the germline. We have already identified several candidate duplications that when present in a diploid dose appear to cause morphological deformities, severe depletion of the germline through germline stem cell loss and differentiation defects. We will determine whether these duplications regulate *Sxl* expression. We are also studying the *Sxl* promoter to identify *cis*-regulatory elements that control expression in the female germline. Investigating the *trans*- and *cis*- regulation of *Sxl* in the germline will allow us to understand how the 'sex' is coordinated to match in the germline and soma. Sometimes a disparity in the sexes can arise, leading to gametogenesis defects, also commonly found in humans. Our study is hence of crucial importance to understanding reproductive biology and human health.

415C

Sexual differentiation of the *Drosophila* germline stem cell niche regulated by *doublesex* and *fruitless*. Hong Zhou, Cale Whitworth, Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore, MD.

Sexual differentiation of the gonad is genetically control by the sex determination pathway in *Drosophila melanogaster*. A key step in establishing gonadal sex identity is the formation of sexually-dimorphic germline stem cell (GSC) niche. Yet little is known downstream of *doublesex (dsx)* about how male-specific differentiation and maintenance is controlled. We recently identified that *fruitless (fru)* is male-specifically expressed in the somatic GSC niche and colocalizes with *doublesex*. In the nervous system, sex-specific FRU expression is regulated by alternative splicing mediated by TRA/TRA-2. However, in the gonad FRU expression is independent of TRA/TRA-2 mediated alternative splicing. Surprisingly, the male form of *dsx* is necessary and sufficient to turn on FRU expression. Our genomic analysis further supports that *fru* is directly targeted by DSX. To investigate the role *fru* plays during male GSC niche differentiation and maintenance, we are conducting gain-of-function and loss-of-function studies in both male and female gonads. We observed defects in female gonads that ectopically expressed *fru*, as well as in testes that knocked down *fru* by RNA interference. We are in the process of analyzing the cause of these defects. We propose based on our preliminary data that *fru* functions downstream of *dsx* in the male gonad as a masculinizing factor to establish and maintain the male niche identity. Since*tra* is less evolutionary conserved than *dsx* in the sex determination hierarchy, the novel sex-specific *fru* expression controlled by *dsx* at transcriptional level may represent a more ancient relationship between *dsx* and *fru,* thus providing insights into the crosstalk between the two sex regulators in the nervous system.

416A

Transfer and processing of the seminal fluid protein Antares during and after mating in *Drosophila melanogaster*. Kaitlyn Baranowski[1], Mariana Wolfner[2], Geoffrey Findlay[1,2]. 1) Department of Biology, College of the Holy Cross, Worcester, MA; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Drosophila females undergo dramatic changes in physiology and behavior after mating. These changes are caused by the receipt of seminal fluid proteins from males. One of these proteins, Sex Peptide (SP), affects male and female reproductive success by inducing egg production and decreasing female remating behavior. These changes last for several days because SP binds to sperm that are stored by the female. SP acts as part of a network of male and female reproductive proteins called the SP network. One of the proteins involved in this pathway is antares (CG30488). When males do not produce antares, males are unable to transfer critical components of the SP network to female. Consequently, SP is unable to be stored on sperm in the days following mating, and mated females exhibit a decrease in egg production and are receptive to remating. To evaluate how antares fits into the SP pathway, we developed an antibody to the antares protein. Using Western blots, we found that antares does not undergo proteolytic processing in mated females and that the protein appears to be stable in mated female reproductive tracts for several hours after mating. We then used RNAi to systematically silence each additional seminal fluid protein in the SP network, which allowed us to observe the effects of their absence on antares. We observed that the transfer of antares from males to females is not impeded by knocking down any other member of the SP network. These results confirm that antares acts at the upstream end of the pathway to facilitate the transfer of other seminal proteins to females. Current work is focused on determining where in the male reproductive tract antares is expressed and how antares physically interacts with other SP network proteins to facilitate their transfer to females.

417B

Interactions between *akirin* and *pannier* during embryonic myogenesis. Dyana C Brooks, Emily R Champion, Scott J Nowak. Department of Biology and Physics, Kennesaw State University, Kennesaw, GA.

The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal and muscle transcription regulator is a key event in the formation of the *Drosophila* larval body wall musculature. However, despite the primary importance of myoblast specification and differentiation for building and patterning the musculature, the identities of many molecular players in this process remain unknown. We have recently identified the highly conserved nuclear protein Akirin as a Twist-interacting factor that appears to play a critical role in the regulation of Twist-dependent gene expression during myogenesis. Using a double heterozygote genetic interaction screen, we have identified *pannier* as a locus that genetically interacts with *akirin* during embryonic myogenesis, despite no known myogenic defects in *pannier* homozygotes. Such an interaction potentially implicates other gene expression processes such as chromatin remodeling during *pannier* expression. By identifying such novel interactions between genes

such as *akirin* and *pannier,* we will obtain crucial insight into Akirin's mechanism of molecular action during myoblast specification and muscle patterning.

418C

Examination of the Roles of RhoGEF2 and Ribbon in Gonad Morphogenesis. Fatma B Ciftci, Jennifer Jemc. Loyola University Chicago, Chicago, IL.

Cell-cell interactions regulate many aspects of tissue morphogenesis, including tissue function, architecture, differentiation and proliferation. In the developing gonad, migration and interaction of the germ cells (GCs) and somatic gonadal precursor cells (SGPs) is critical for creating a functional organ with the proper architecture. We have identified two genes, *ribbon* (*rib*) and *Rho guanine exchange factor 2* (*RhoGEF2*), that are required for embryonic gonad morphogenesis. Mutations in *rib* and *RhoGEF2* exhibit defects in SGP-SGP interactions and between SGP-GC interactions, both of which are critical during gonad formation. However, the roles of *RhoGEF2* and *rib* during gonad development, and the relationship of these genes to each other are not well understood. Previous work suggests that Rib functions as a transcriptional regulator, while Rho family proteins regulate the actin cytoskeleton. Studies of salivary gland morphogenesis suggest that Rho1 and Rib cooperate in this developmental context. In order to understand the relationship of these genes during gonad morphogenesis genetic analysis was performed. Analysis of double heterozygotes reveals *rib* and *RhoGEF2* double heterozygous mutants have an increased number of defects in gonad morphogenesis relative to controls. As changes in the cytoskeleton are critical for organogenesis, we hypothesize that Rib may regulate transcription of Rho family proteins to promote proper organ formation.

419A

The smooth muscles cells of the *Drosophila* testes arise by myoblast fusion. Katharina Fritzen, Jessica Kuckwa, Renate Renkawitz-Pohl. Philipps-University, Marburg, Hessen, Germany.

The male reproductive system comprises pairs of testes, seminal vesicles, paragonia (also known as accessory glands), and one ejaculatory duct with an annexed sperm pump and the external genitalia. The testes develop from the gonads, which are already formed during embryogenesis, whereas all other parts arise from the male genital imaginal disc which assembles during larval development. During metamorphosis the genital disc and the gonads start to grow towards each other until epithelial cells of the seminal vesicle fuse with the terminal epithelium of the testes. The myoblasts, which will form the muscles sheaths of all reproductive organs, start to migrate onto the testes after attachment. Interestingly the muscle sheaths of the different organs of the male reproductive system vary in appearance although they all arise from the same stem cell pool. The paragonia and the ejaculatory duct are encircled by mononucleated, striated myotubes, whereas the seminal vesicles and the sperm pump contain multinucleated, striated muscles. The musculature of the paragonia and the ejaculatory duct resemble the muscles of the *Drosophila melanogaster* ovaries, where the first mononucleated striated muscle were described. Surrounding the testes is a multinucleated muscle layer which resembles the smooth muscles of vertebrates. The nascent myotubes forming the muscle sheath are multinucleated before they migrate from the genital imaginal disc onto the testes. This previously unknown type of musculature evoked developmental interest. Is the multinucleation achieved by myoblast fusion, as described for other myogenic processes, or do the myoblasts undergo incomplete mitosis without cytokinesis? An EdU incorporation assay revealed that no replication can be detected in myoblasts of the genital imaginal disc after 28h APF, where we observe first multinucleated cells. At this time, expression of the well-studied myoblast fusion relevant adhesion molecules 'Dumfounded' and 'Sticks and Stones' can be observed in the myoblasts of the genital imaginal disc. Thus we hypothesise that the smooth multinucleated myotubes of the testes arise by myoblast fusion on the genital imaginal disc.

420B

Noncanonical roles for Tropomyosin during myogenesis. Jessica Williams, Nathan Boin, Juliana Valera, **Aaron Johnson**. Dept Integrative Biology, Univ Colorado, Denver, Denver, CO.

For skeletal muscle to produce movement, individual myofibers must form stable contacts with tendon cells and then assemble sarcomeres. The myofiber precursor is the nascent myotube, and during myogenesis the myotube completes guided elongation to reach its target tendons. Although myotube elongation is essential for muscle function, the molecules that direct this process remain largely unknown.*hoi polloi (hoip)* encodes a highly-conserved RNA binding protein and *hoip* mutant embryos are largely paralytic due to defects in myotube elongation and sarcomeric protein expression. We used the *hoip*mutant background as a platform to identify novel regulators of myogenesis, and uncovered surprising developmental functions for the sarcomeric protein Tropomyosin 2 (Tm2). Tm2 overexpression rescued the *hoip* myogenic phenotype by first stabilizing F-actin at the myotube leading edge during elongation, and second by promoting the expression of other sarcomeric RNAs after elongation initiates. Thus, prior to regulating contraction in the sarcomere, Tm2 dictates fundamental steps of myogenesis. .

421C

The Role of the BTB Family Proteins Lola and Rib in Gonad Morphogenesis. Diane Silva, Christopher P. Lenkeit, Edwin Chaharbakhshi, Andrew Droste, Jennifer Jemc. Loyola University, Chicago, IL.

During organogenesis the gonad is formed from two primary cell types, the somatic gonadal precursors (SGPs), and the germ cells (GCs). When SGPs and GCs fail to come together in the gonad they exhibit fusion defects, and when they fail to form a spherical structure they exhibit compaction defects. Two genes identified to exhibit these defects during gonad morphogenesis in *Drosophila melanogaster*are *longitudinals lacking (lola)* and *ribbon (rib)*. Both RIB and LOLA contain a BTB (Broad-complex, Tramtrack and Bric-á-

brac) domain, which is a protein-protein interaction domain, suggesting that these proteins may be functioning together in gonad morphogenesis, as has been observed for other BTB domain containing proteins. Most BTB domain proteins also frequently contain DNA binding domains, and RIB and LOLA do as well, suggesting they may regulate transcription of target genes. We have found that RIB and LOLA are expressed in the *Drosophila* embryonic gonad and would like to further understand if they are cooperating to regulate gene transcription during gonad development. Through a genetic interaction study we have found that there is an increase in fusion and compaction defects in *rib/lola* double heterozygotes relative to controls, suggesting that they function together to regulate gonad development. Current studies are examining a potential physical interaction between these proteins through yeast-two-hybrid and immunoprecipitation. Given that Rib and Lola are transcription factors, we are also working to identify the downstream targets through which these proteins function to regulate the migration and interaction of SGPs and GCs during gonad development. Rib and Lola are known to regulate morphological changes in multiple developmental contexts, and these studies will provide insight into the mechanisms by which they do so.

422A

Transcriptome analysis of embryonic tendon and muscle cells during myotube elongation. Juliana Valera, Aaron Johnson. Department of Integrative Biology, University of Colorado Denver, Denver, CO.

Myotube elongation is the process by which nascent myotubes extend and identify appropriate tendon attachment sites. Although myotube elongation is an essential component of muscle morphogenesis and diversification, the molecular pathways that guide nascent myotubes toward tendon cells remain largely unknown. At the morphological level, tendon progenitor cell loss causes inappropriate myotube localization suggesting that tendon cells secrete essential myotube guidance cues. To identify these guidance pathways, we purified populations of embryonic tendon cells and nascent myotubes by fluorescence activated cell sorting (FACS). We isolated cell-type specific mRNAs from these cell populations and have submitted the samples for RNA-seq. We expect that our analysis of the sequencing data will identify novel myotube guidance pathways and will provide unique insights into the molecular mechanisms that regulate myogenesis.

423B

Genetic Analysis of Conserved Eya Protein Domains in *Drosophila*. M. Jin, G. Mardon. Pathology Dept, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030.

Although the *eyes absent* (*eya*) gene plays vital roles in multiple aspects of *Drosophila* eye development, many questions remain about the mechanisms of *eya* action *in vivo*. Our goal is to evaluate the requirement for conserved Eya protein domains by using a genomic rescue (GR) system and site-specific integration so that different constructs can be directly compared. Eya proteins contain a PST (Proline-Serine-Threonine)-rich transcriptional activation domain, two protein phosphatase domains (tyrosine and threonine), two MAP kinase (MAPK) phosphorylation sites, and an 'eh1' motif that mediates binding to the corepressor Groucho. We have made a series of genomic rescue constructs that carry combinations of mutations affecting these domains and have tested their ability to rescue *eya* eye-specific and null mutations. Our previous studies have shown that the two MAPK phosphorylation sites and the putative tyrosine phosphatase activity of Eya appear to be dispensable for normal Eya function. However, we do observe strong phenotypes with a point mutation in the eh1 motif (*eya*^{eh1}*GR*), deletion of the PST/threonine phosphatase (TP) domain (*eya*$^{Del\ PST/TP}$*GR*), four active site point mutations in the TP domain (*eya*^{Y4}*GR*), and when the PST domain is replaced by the heterologous VP16 activation domain (*eya*$^{Del\ PST/TP+VP16}$*GR*). Additional constructs are underway to further decipher the roles of these domains *in vivo*. Our analyses will also include genome-wide expression profiling of eye imaginal disc RNA at specific time points to evaluate the consequences of these mutations *in vivo*. Together with the extensive RNA sequencing we have conducted on many other genotypes, these data will provide an unprecedented understanding of the molecular mechanism of Eya function.

424C

Signaling through Rickets, a G-protein-coupled receptor, is crucial for polarity and migration of the border cells in *Drosophila*. Lauren Anllo, Trudi Schupbach. Dept. of Molecular Biology, PRINCETON UNIVERSITY, PRINCETON, NJ.

Cell migration plays crucial roles during development, for instance during gastrulation, nervous system development and organ formation. Research clarifying the mechanisms that regulate cell motility in normal developing systems also has clinical relevance to understanding the nature of tumor metastasis. Migration of border cell clusters within a developing *Drosophila* egg chamber provides an excellent model to study coordinated cell movements. A *Drosophila* egg chamber is comprised of a single layer of somatic follicular epithelial cells surrounding 16 germline-derived cells. During oogenesis, a cluster of cells within the anterior follicular epithelium becomes specified as border cells, and delaminates from the epithelium. In this process the cells lose their normal epithelial morphology and undergo an shift in polarity organization. This cluster then extends filopodia toward the oocyte and the cells migrate as a group between germline cells of the developing egg chamber to reach the oocyte at the egg chamber's posterior end. In an EMS mutagenesis screen on chromosome 2L performed to study general follicle cell development, we isolated two alleles of the gene *rickets* (*rk*) – encoding a G protein coupled receptor. The *rk* alleles result in border cell migration defects in a significant fraction of egg chambers. We used the FLP/FRT system to generate mosaic egg chambers containing cells with homozygous mutations for *rk* alleles. RNAi against *rk*, and its ligand *burs* was also utilized. In *rk* mutants, border cells are properly specified and express the marker Slbo. Yet some single border cells tend to lag behind the main border cell cluster during migration, and in some cases, the entire cluster does not detach from the anterior, or only migrates part of the distance. The border cells also show defects in localization of various apical polarity markers during migration. Our screen has thus identified a receptor with a previously unknown role in border cell migration that

appears to regulate polarity and detachment of the border cell cluster, allowing it to migrate appropriately.

425A

Exploring the Role of Raw in the Embryonic Nervous System. Emily R. Temple-Wood, Diane Silva, Jennifer Jemc. Department of Biology, Loyola University Chicago, Chicago, IL, IL.

Previous studies have demonstrated that Raw function is required in multiple developmental contexts, including dorsal closure, salivary gland and malpighian tubule morphogenesis, and gonadogenesis. Studies of the role of Raw in gonad morphogenesis demonstrates that Raw promotes proper cell-cell interactions via its regulation of cell adhesion proteins and the JNK signaling pathway. Therefore, we are interested in the role of Raw in mediating cell-cell interactions in other tissues. Recent work in our lab suggests that Raw is required in the glia of the peripheral nervous system in the third instar larva and may play a role in mediating glia-neuron interactions at this stage in development. However, evidence suggests that Raw is required for nervous system development beginning during embryogenesis. Immunohistochemistry reveals that Raw is expressed in the developing nervous system in the embryo, and previous work suggests that it is required for CNS retraction during embryogenesis, leading us to hypothesize that Raw plays a critical role in glia-neuron interactions from embryogenesis onward. These studies examine the effects of mutating *raw* on glia specification and migration, as well as axon guidance.

426B

Dynamic protein modification: Variable tissue-specific modification and localization of eRpL22-like during fly eye development. Brett Gershman, Michael Kearse, Vassie Ware. Biological Sciences, Lehigh University, Bethlehem, PA.

The ribosomal protein paralog eRpL22-like is expressed in a tissue-specific manner and is differentially post-translationally modified within the fruit fly testis and eye. In testis, eRpL22-like is not modified (34kD) but in the eye, eRpL22-like is detected both as an unmodified species as well as a higher molecular mass (mm) species (60kD). Several lines of evidence [including co-immunodetection with eRpL22-like or *Drosophila* SUMO antibodies, reciprocal co-immunoprecipitation against eRpL22-like or SUMO, and *in vivo* RNAi for eye-specific depletion of SUMO (Smt3)] support a role for SUMOylation in generating eye-specific higher mm eRpL22-like. The functional significance of modified eRpL22-like is unknown, but ribosome profiling of head tissue shows that modified eRpL22-like is not a component of translating ribosomes, suggesting an extraribosomal role. We are interested in exploring the developmental history of eRpL22-like modification within the fly eye to determine if eRpL22-like function may vary during the course of fly eye development. Three main developmental stages (third instar, midpupa and adult) were screened to determine the pattern of eRpL22-like expression and modification. Western blot analysis shows that eRpL22-like migrates at ~38kD in third instar larval eye/antennal imaginal discs, suggesting eRpL22-like protein modification. In midpupal retinas, eRpL22-like is unmodified. Based on this modification pattern, we propose that high mm modification to 60kD in the eye occurs between midpupal and adult stages. Immunohistochemistry shows that eRpL22-like localization differs at each developmental stage, with varying protein abundance in different eye regions and eRpL22-like exclusion from areas of the presumptive ommatidia. Whether or not eRpL22-like in third instar eye/antennal imaginal discs or in midpupal retinas is associated with actively translating ribosomes remains to be determined. Polysome profile analysis of eye tissue from these developmental stages will inform our understanding of potential differences in the function of eRpL22-like moieties. These studies may provide insights into the role of the temporally dynamic modification pattern of eRpL22-like in fly eye development.

427C

Kette and WASP act antagonistically during F-actin formation in fusion competent myoblasts. A. Loewer[1], G. Schaefer[2], J. Hamp[3], S. Oenel[1]. 1) Entwicklungsbiologie, Philipps-Universitaet Marburg, Marburg, Germany; 2) Institute for Genetics, Marburg, Germany; 3) Fresenius Kabi GmbH, Bad Hersfeld, Germany.

The formation of skeletal muscles depends on intercellular cell-cell fusion composed of mutual recognition and adhesion between myoblasts resulting in membrane fusion. In *Drosophila* two types of myoblasts, the so called founder cells (FCs) and fusion competent myoblasts (FCMs) fuse to form a mature muscle (reviewed in Abmayr and Pavlath, 2013). Both cell types show different shapes and behaviors during the fusion process especially in formation of F-actin at the site of fusion. While FCs show a rather thin actin sheath a thick actin focus can be observed in FCMs, indicating a difference in their regulation of generation. Essential for their formation is the F-actin nucleating Arp2/3 complex. Extensive studies on this Arp2/3 based actin polymerization during myoblast fusion have shown that the activation of the Arp2/3 complex depends on the nucleation promoting factor (NPF) SCAR (WAVE) in FCs and on the activation of both NPFs SCAR and WASP (Wiskott-Aldrich syndrome protein) in FCMs. The activity of SCAR is controlled by the SCAR complex which consists of 4 proteins including Kette (Hem-2/Nap-1). The loss of *kette* results in severe myoblast fusion defects in homozygous null mutant embryos. Recently, we generated *kette wasp* double mutants (Schäfer et al. 2007). Homozygous *kette wasp* double mutant embryos display a *kette*-like phenotype. However we observed a suppression of the *kette* mutant phenotype when the *wasp* gene dosage was reduced. This suggests an antagonizing effect of Kette on the activity of the WASP pathway. Here we present first genetic experiments to determine the coordination of *scar* and *wasp* dependent Arp2/3 activation in FCMs.

428A

Loh: a matricellular protein required for cardiac function. Bárbara Rotstein[1], Maik Drechsler[2], Ariane Wilmes[1], Achim Paululat[1]. 1) Osnabrueck University, Department of Developmental Biology, Osnabrueck, Germany; 2) University of Cambridge, Department of Zoology, Cambridge, England.

Matricellular proteins are extracellular proteins that bridge between matrix proteins and cell surface receptors, or other molecules

such as cytokines and proteases that interact, in turn, with the cell surface. Qualitative and quantitative alterations in matricellular proteins (Volk, et al., 2014) are often associated with different pathologies, including cardiac diseases. We use the dorsal vessel (the heart) of*Drosophila melanogaster* as a model to investigate general aspects of cardiac extracellular matrix (ECM) composition, deposition and assembly in a genetically treatable system. Aim is to understand how certain ECM components of the cardiac ECM contribute to the establishment of specific biomechanical properties, thereby facilitating coordinated organ function.
In a genetic screen for genes mediating the development of the embryonic heart, our lab identified Lonely heart (Loh), an ADAMTS-like protein that directs heart-specific localization of the ECM protein Pericardin (Prc) (Drechsler et al., 2013). In order to elucidate on how Loh recruits Prc, we have designed an *in vivo* Prc recruitment assay based on the ectopic expression of mutated Loh versions in tissues, where loh is normally not expressed (e.g. muscle cells). Our results clearly indicate that Loh is essential for the assembly and accumulation of the Prc protein. But it is still unclear whether Loh performs the function through a direct or indirect interaction with Prc. In order to distinguish between these hypotheses, we are performing interaction assays, as well as biochemical and bioinformatics approaches. To identify the functional domains in Loh required for its adhesion to the cardiac matrix, we plan to express full-length and mutated forms of Loh, all Myc-tagged, in transgenic *Drosophila* or in S2/Kc cells. All together, our studies aim to provide a better understanding on ADAMTS-like proteins mechanics in cardiac matrices.

429B

Dynamic Notch signaling sequentially specifies cell fate in the secretory lineage of *Drosophila* spermathecae. Wei Shen[1], Fabio Carvalho[1], Jianjun Sun[1,2]. 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs.

Type-III insect glands are one class of exocrine glands that regulates multiple aspects of insect physiology and behavior. Well-known examples include the spermathecal gland, silk-producing labial gland, bee-sting gland and insect epidermal glands. Ultra-structural study of these glands has demonstrated the common organization of each secretory unit, in which each secretory cell is connected to the central lumen *via* a cuticular end apparatus and canal that acts as a secretion reservoir and discharging duct, respectively. However, how the secretory unit is built is largely unknown. Using *Drosophila*spermathecae as a model, we recently uncovered the essential role of nuclear hormone receptor Hr39 in spermathecae formation and the physiological function of glandular secretions in ovulation and sperm storage. We also defined the secretory cell lineage in which each secretory unit precursor (SUP) divides sequentially to give rise to an apical cell (AC), a secretory cell (SC), and a basal cell (BC), which together give rise to an adult secretory unit. No role has been assigned to each cell and the signals that specify each cell type's fate is also unknown. Here we investigated the role of Notch signaling in spermathecae formation. Notch is dynamically activated in the secretory lineage, such that it is activated in lumen epithelial precursors (LEPs) 24 hour after puparium formation (24h APF) and in secretory cells (SCs) 48h APF. Loss of Notch signaling transforms LEPs into SUPs and SCs into BCs, while gain of Notch signaling transforms SUPs into LEPs and BCs into SCs. In the latter case, the secretory unit with two SCs can no longer orient their end apparatuses at the apical side, indicating that BC is not required for end apparatus formation but is essential for correct orientation. Our work, for the first time, demonstrates a role for Notch signaling in cell fate determination in the spermathecal lineage and uncovered an essential role for BCs in secretory unit formation.

430C

Genes expressed in the secondary cells of the male accessory gland are essential for the female post mating response. Jessica Sitnik[1], Dragan Gligorov[2], Robert Maeda[2], Fancois Karch[2], Mariana Wolfner[1]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Genetics and Evolution and NCCR Frontiers in Genetics, University of Geneva, Geneva, Switzerland.

Seminal proteins from the Drosophila male accessory gland induce post-mating responses (PMR) in females. The PMR comprises behavioral and physiological changes that include increased egg-laying, decreased receptivity to courting males, and changes in storage and use of sperm. Many of these changes are induced by a "sex peptide" (SP), and are maintained by SP's binding to, and slow-release from, sperm. The accessory gland contains two secretory cell types whose morphology and development differs. Products of these "main" and "secondary" cells work interdependently to induce and maintain the PMR. Main cells make SP and some of the molecules that bind it to sperm; the large highly vacuolated secondary cells make other parts of the machinery that binds SP to sperm.
To identify genes needed for the morphology and function of secondary cells, we studied *iab6[cocu]* males. Because of lack of *Abdominal-B (Abd-B)* in secondary cells of these males, those cells have abnormal morphology and fail to provide products to maintain the PMR. By RNA-seq we identified 73 genes whose expression is down-regulated in *iab6[cocu]*males. By functional assays and microscopy we found at least 10 of these genes that are required for normal storage and release of SP in mated females. Interestingly only 1 of those genes encodes a previously reported seminal protein, suggesting that secondary cells may perform essential functions beyond the production of known Sfps. At least 3 of the genes regulate the size and/or abundance of secondary cell vacuoles, suggesting that the vacuoles' contents are important for the machinery for maintaining the PMR.

431A

Investigatingon of the Roles of Mrityu in Egg Activation in *Drosophila melanogaster*. Zijing Zhang[1], Amber Krauchunas[2], Mariana Wolfner[1]. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Waksman Institute of Microbiology, Rutgers University, New Brunswick, NJ.

Egg activation is essential for the successful transition from a mature oocyte to a developmentally competent egg. It consists of a series of events including the resumption and completion of meiosis, initiation of translation of some maternal mRNAs, and changes to

the vitelline envelope. This drastic change of cell state is accompanied by large scale alteration of phosphoproteome of the cell. Previous studies in our lab identified 311 proteins that are phospho-regulated during egg activation, which we hypothesize represent an enriched set of candidates whose activities regulate egg activation (Krauchunas, Horner et al. 2012). Our ongoing RNAi screen of these candidates for roles in egg activation has identified 28 genes whose germline knockdown results in eggs with no or low hatchability. Initial screens through these genes identify *Mrityu (Mri)*, *Bre1* and *Mod(mdg4)* as potential new regulators of egg activation or initiation of embryogenesis. For example, maternal RNAi knockdown of Mri causes fertilized eggs to arrest at metaphase of the first two mitotic cycles. We find that Mri is phosphorylated upon egg activation. A study of the mammalian ortholog of Mri, BTBD10, proposed a novel interaction between BTBD10 and PP2A-C, suggesing the possibility that an analogous interaction exists between Mri and *Drosophila* PP2A-C (Nawa, Kanekura et al. 2008). We are testing this by creating and examining mutations in Mri, and knockdown in PP2A-C, for phenotypes, and by biochemical approaches.

432B

Investigating the role of *Hrb27C* in the regulation of female germline stem cell activity. Danielle Finger, **Elizabeth Ables**. Dept. of Biology, East Carolina University, Greenville, NC.

Stem cells incorporate a variety of intrinsic and extrinsic cues to maintain their fate and proliferative capacity. Importantly, stem cells in many tissues are regulated by steroid hormone signaling. Although the biological actions of steroid hormones in human tissues are well-characterized, the full repertoire of steroid-dependent transcriptional networks controlling these actions in stem cells remains largely undescribed. The *Drosophila melanogaster* female germline provides the ideal model to study the mechanisms of steroid hormone signaling *in vivo*. *Drosophila* oogenesis is maintained by the asymmetrical division of germline stem cells (GSCs), which are regulated by both local signaling from adjacent somatic cap cells and endocrine signaling by ecdysone, a prototypical steroid hormone structurally similar to mammalian sex steroids. Genome-wide studies identified *Heterogeneous nuclear ribonucleoprotein at 27C (Hrb27C*) as a putative target of ecdysone signaling during development. Hrb27C is an abundant heterogeneous nuclear ribonuclear protein required in the developing oocyte for the proper localization of critical axis-determining transcripts. We have confirmed that *Hrb27C* is widely expressed in the germline and soma throughout oogenesis, including in GSCs. Our preliminary results, using the *FLP/FRT* technique to generate genetic mosaics, indicate that *Hrb27C* mutant GSCs fail to be maintained in the niche, suggesting that *Hrb27C* is required for GSC activity. Taken together with published data, we hypothesize that ecdysone signaling promotes *Hrb27C* expression to regulate GSC activity. Given the conservation between steroid hormone signaling in *Drosophila* and mammals, our studies may also help us understand the targets of steroid hormone signaling in mammalian tissue-resident stem cells.

433C

Identification of Germline sex defining signals in *Drosophila melanogaster*. Pradeep K. Bhaskar, Raghav Goyal, Kelly Baxter, Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore, MD.

Sexual dimorphism is common throughout animal kingdom; males and females exhibit phenotypic characters specific for their "sex". It has been established that not only somatic cells but the germ cells also determine their sex. The establishment of germ cell sexual identity is critical for sex-specific development of germline stem cells and production of sperm vs. eggs. Thus, it is an essential aspect of animal sexual reproduction and human fertility. Germ cells depend on both signals from the somatic gonad as well as their own sex chromosome genotype to determine their sex. Therefore, when the "sex" the germline fails to match the "sex" of the soma, germline development is severely disrupted. How somatic signals and germ cell intrinsic cues act together to regulate germline sex determination is a key question about which little is known in any organism. Previously, we identified PHF7 as a protein highly biased to the male germline that is required in male germ cells and toxic to female germ cells. Our data indicate that PHF7 is regulated at both the transcriptional and translational levels to control male-specific expression. RNAseq data indicates that *Phf7* is expressed from different promoters in males and females, and that only the male transcript is efficiently translated. The *Phf7* male-specific promoter has two STAT binding sites, indicating that it may be regulated by JAK/STAT signaling from the soma to the germline, and our initial experiments support this hypothesis. We currently analyzing whether *Phf7* is a direct STAT target gene. Further, since our data indicate that the sex determination factor Sex-lethal regulates the JAK/STAT pathway in the germline, we propose that SXL, JAK/STAT and PHF7 act in a coordinated manner to mediate germline sex determination.

434A

Niche signaling promotes stem cell survival in the *Drosophila* testis via the Jak-STAT target DIAP1. Salman Hasan[1], Phylis Hetie[1,2], Dr Erika Matunis[1]. 1) Johns Hopkins Medical Institute, Baltimore, MD; 2) Harvard University, Cambridge, MA.

Tissue-specific stem cells are thought to resist environmental insults better than their differentiating progeny, but this varies from one tissue to another, and the underlying mechanisms are not well-understood. Here, we use the *Drosophila* testis as a model system to study the regulation of cell death within an intact niche. This niche contains sperm-producing germline stem cells (GSCs), and accompanying somatic cyst stem cells (or CySCs). Although many signals are known to promote stem cell self-renewal in this tissue, including the highly conserved Jak-STAT pathway, the response of these stem cells to potential death-inducing signals, and factors promoting stem cell survival, have not been characterized. Here we find that both GSCs and CySCs resist cell death better than their differentiating progeny, under normal laboratory conditions and in response to potential death-inducing stimuli such as irradiation or starvation. To ask what might be promoting stem cell survival, we characterized the role of the anti-apoptotic gene *Drosophila* inhibitor of apoptosis 1 (*diap1)* in testis stem cells. DIAP1 protein is enriched in the GSCs and CySCs, and is a Jak-STAT target. *diap1* is necessary for survival of both GSCs and CySCs, and ectopic up-regulation of DIAP1 in somatic cyst cells is sufficient to non-autonomously rescue

stress-induced cell death in adjacent differentiating germ cells (spermatogonia). Altogether, our results show that niche signals can promote stem cell survival by up-regulation of highly conserved anti-apoptotic proteins, and suggest that this strategy may underlie the ability of stem cells to resist death more generally. .

435B

Aging delays the S phase progression of normal and tumorous germline stem cell division cycles distinct from the effect of diet. Shih-Han Kao[1], Chen-Yuan Tseng[1,2], Chih-Lin Wan[1], Yu-Han Su[1], Hwei-Jan Hsu[1]. 1) Insitute of Cellular and Organismic Biology, Academia Sinica 128 Academia Road, Section 2, Nankang, Taipei 11529, Taiwan, R.O.C; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan.

Stem cell function declines with age, but very little is known about the mechanisms involved. It has been shown that aging attenuates insulin signaling, which controls the G2 phase of the *Drosophila* female germline stem cell (GSC) division cycle and GSC maintenance in response to diet. It is not clear, however, if aging also controls GSC division via insulin signaling, and if GSC division capacity contributes to its maintenance. Here we show that tumorous GSCs are accumulated, while GSC number and proliferation rate decrease in both mated and non-mated female flies with age. These results indicate that aging is linked to ovarian cancer risk and mating *per se* does not affect stem cell function with age. Intriguingly, accelerated or slowed GSC division rate results in GSC loss, suggesting the contribution of GSC division rate to age-dependent GSC loss. Furthermore, DNA damage accumulates in GSCs and the S phase of the GSC cell cycle is predominately prolonged with age. In addition, GSC tumors, which escape the normal stem cell regulatory microenvironment, or niche, still respond to aging in a similar manner to normal GSCs, indicating that niche signals are not required for GSCs to sense or respond to aging. Our results document the impact of aging and diet on stem cell division cycle are distinct and also demonstrate the complexity of aging effects on stem cells.

436C

Heparan sulfate proteoglycans regulate germline stem cell behavior and niche organization in the *Drosophila* testis. Daniel C Levings[1], Hiroshi Nakato[2]. 1) Molecular, Cellular, Developmental Biology and Genetics Program, University of Minnesota-Twin Cities, Minneapolis, MN; 2) Department of Genetics, Cell Biology and Development, University of Minnesota-Twin Cities, Minneapolis, MN.

Stem cell niches are discrete microenvironments where stem cell proliferation is tightly regulated via short-range signals from nearby niche structures. These niches allow for many of the behaviors that uniquely characterize stem cells, including their ability to self-renew and their ability to asymmetrically divide, producing one stem cell and one differentiating daughter cell. As a classical model stem cell system, the male germline stem cell (GSC) niche in the *Drosophila* testis is particularly well-suited for studying such stem cell behaviors. We are especially interested in how a class of extracellular, carbohydrate modified proteins, heparan sulfate proteoglycans (HSPGs), regulate these behaviors. We found that complete loss of heparan sulfate (HS) chains results in collapse of the germline, indicating a role for HS in GSC maintenance. Further experiments revealed, however, that partial loss of HS sulfation or loss of sulfation specifically in the somatic testis niche structure, the hub, leads to increased GSC numbers, defects in the asymmetric division process, and the appearance of ectopic stem cells outside the niche. Specifically, we found that RNAi knockdown of HS sulfation in the hub results in defects in the stereotypical centrosome orientation seen in wild type GSCs and in the formation of ectopic populations of GSCs and cyst stem cells. These results suggest that HSPGs are important in coordinating niche-stem cell interactions and that changes in HS sulfation levels can act as a mechanism for fine tuning of niche biology. .

437A

Loss of the Nuclear Lamina protein Otefin reveals a novelgermline stem cell checkpoint . Kaylee E. Lovander, Lacy Barton, James Bullard, Pamela Geyer. Department of Biochemistry, University of Iowa, Iowa City, IA.

Many tissues are maintained by stem cells. Emerging evidence suggests that adult stem cell populations require nuclear lamina proteins for homeostasis and function. To understand how nuclear lamina proteins maintain adult stem cells, we study the Drosophila Emerin homologue, Otefin (Ote). Loss of Ote causes male and female sterility, resulting in a complex mutant phenotype that includes germline stem cell (GSC) loss and niche defects. Using developmental and tissue-restricted expression analyses, we established that Ote is intrinsically required in GSCs for germline differentiation and GSC survival. Interestingly, we find that loss of Ote activates the DNA Damage Response (DDR) transducer Checkpoint kinase 2 (Chk2). Activation of this kinase causes $ote^{-/-}$ GSC loss, demonstrated by findings that$ote^{-/-}$,$chk2^{-/-}$ GSCs are maintained and give rise to daughters that undergo all stages of oogenesis. Investigations of the epistatic relationships between *ote* and other DDR genes revealed that loss of ATR, but not ATM or p53, suppresses loss of $ote^{-/-}$ GSCs. Although members of the DDR pathway are activated, immunohistochemical analyses failed to detect DNA damage. As uncapped telomeres are linked to DDR activation, we investigated telomere structure, finding that telomere structure and number are unchanged in $ote^{-/-}$ GSCs. These data imply that a non-canonical trigger activates Chk2. Investigations into possible causes revealed a connection to nuclear architecture, as $ote^{-/-}$ GSCs show large aggregates of lamin Dm0 with the nuclear envelope and increased staining of heterochromatic markers. Both defects persist in $ote^{-/-}$,$chk2^{-/-}$ GSCs, indicating these changes are upstream of Chk2 activation. Taken together, our studies suggest the presence of a novel GSC survival checkpoint that monitors nuclear architecture.

438B

Wnt signaling regulates escort cell-expressed Thickveins to constrain Dpp activity within the Drosophila ovarian stem cell niche. Lichao Luo[1,2], Huashan Wang[1], Chao Fan[1]. 1) Temasek Life Science Laboratory, Singapore, Singapore, 117604; 2) Department of Biological Sciences, National University of Singapore, Singapore, 117543.

Reproduction needs a well-regulated system of germ stem cells (GSCs) which is able to balance self-renewal and differentiation. Both the pro-differentiated activity and the tumorigenesis caused by over proliferation could lead to deficiency of egg/sperm production. To maintain to GSCs, many organisms provide an anatomical niche surrounding stem cells where signals and physical support are used to mediate GSCs cell fate. In the Drosophila female germline stem cell niche, Decapentaplegic (Dpp), a fly TGFβ and well-established long rang morphogen, acts over one cell diameter to maintain the GSCs. Here, we show that Thickveins (Tkv, a type I receptor of Dpp), is highly expressed in stromal cells next to Dpp-producing cells and functions to remove excess Dpp outside the niche, thus spatially restricting its activity. Interestingly, Tkv expression in these stromal cells is regulated by multiple Wnt ligands produced by the niche. Our data demonstrate a self-restraining mechanism by which the Drosophila ovarian GSC niche acts to define its own boundary. This mechanism could be widely deployed in other stem cell niches where multiple signaling pathways are involved.

439C

Up-regulation of germline stem cell division frequency in response to mating. Manashree Malpe, Karl Kudyba, Chun Ng, Cordula Schulz. Cellular Biology, University of Georgia, Athens, GA.

Stem cells act as precursors for a variety of specialized cells in the body. The stem cell microenvironment or niche plays an important role in maintaining stem cell properties and inducing required differentiation pathways. However, the physiological and developmental state of an organism also plays an important role in stem cell function and stem cell daughter differentiation. Our laboratory discovered that males that were heavily mated to virgin females displayed a significant increase in their germ line stem cell (GSC) division frequency compared to their non-mated siblings. To explore this upregulation on molecular level, we generated transcriptomes of testes tips from 6 isogenized (Oregon R and Canton S) wild type strains under both, mated and non-mated conditions. From the data, we have shortlisted about 15 target genes that are differentially expressed in mated versus non-mated males from all 6 strains. We are currently using germline specific knockdown via RNAi to validate and investigate the roles of these target genes.

440A

Gap junction-mediated signalling regulates the proliferation and differentiation of somatic cyst cells in the *Drosophila* testis. Christopher M Smendziuk, Fayeza Islam, Anat Messenberg, Guy Tanentzapf. Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada.

Gametogenesis is a conserved process in animals that requires intricate signalling between germ cells, which will give rise to sperm or eggs, and somatic cells, which support germline development. A key feature of gametogenesis is the involvement of specialized stem cells that give rise to both the soma and the germline. Previous work in *Drosophila* has illustrated that soma-germline interactions control stem cell behaviour. Failure to achieve proper regulation of soma-germline communication within the stem cell niche or during spermatogenesis can result in infertility or the formation of tumours. To better understand the cell-biological underpinnings of how the soma and germline communicate, we used a candidate-gene approach. Flies containing mutations in the gene *zero population growth/innexin4 (zpg)* are sterile and possess tiny gonads. *zpg* has been shown to code for an innexin, a gap junction protein. Previous studies indicate that Zpg functions in the germline to regulate germ cell function but the precise role of Zpg has not yet been elucidated. Our preliminary data support the idea that Zpg mediates communication from the germline to the soma. We have uncovered previously uncharacterized defects in the soma of *zpg* mutants, including overproliferation and delayed differentiation. In addition, we have analysed the function of Innexin2 in the testis, showing that Zpg in the germline interacts with Innexin2 in the soma to regulate spermatogenesis. Our observations support the assertion that Zpg helps form gap junctions between the soma and germline. To further analyse the function of Zpg, we are carrying out a structure/function analysis of the Zpg protein. Altogether, our studies are beginning to provide mechanistic insight into germline-soma communication and the role of gap junctions in regulating stem cell proliferation and differentiation.

441B

Insulin Signaling-Mediated Control of the 'Failed Axon Connections' Protein in the Niche Maintains Germline Stem Cells in Response to Diet. Yu-Han Su, Hwei-Jan Hsu. Institute of Cellular and Organismic Biology, Academia Sinica 128 Academia R.d., Sec 2, ICOB R337, Nankang, Taipei 115, Taiwan, R.O.C.

Stem cells not only respond to their supporting cells (the niche), but also to nutritional inputs, which are required for their proper function; however, not much is known about how these processes are coordinated. Here, we report that *Drosophila* Failed axon connections (Fax) is expressed in the niche, regulated by insulin signaling, and controls ovarian germline stem cell (GSC) identity in response to diet. The *fax* gene has six predicted transcripts, named A to F, while Fax A and C are predominately expressed in the ovary. Fax contains a conserved glutathione S-transferase (GST) N-terminal and a GST-C-terminal domain, and exhibit high similarities with orthologs in vertebrate species. Flies carrying a GST-C terminal domain truncated Fax exhibit less GSCs and niche cap cells (a component of the GSC niche) right after eclosion, while GSC and niche cap cell numbers are not decreased further one week after eclosion; suggesting the requirement of GST-C terminal domain of Fax in the development of GSC-niche unit. In addition, Fax is highly expressed in escort cells that serve as niches for GSC maintenance and germ cell differentiation. Knock down of Fax in adult escort cells causes GSC loss, suggesting that Fax controls GSC maintenance through niche escort cells. Finally, Fax expression in escort cells is reduced in *insulin receptor* mutants and by a protein-poor diet. Although the mechanisms involved need further investigation, our studies have uncovered the role of Fax as a nutrient responder in escort cells, and its function in niche cells in influencing stem cell identity.

442C

Uncovering the role of adipocyte-derived collagen type IV in regulating *Drosophila* oogenesis. Lesley Weaver, Daniela Drummond-Barbosa. Department of Biochemistry and Molecular Biology, Johns Hopkins University, Baltimore, MD.

Inter-organ communication in response to nutritional signals influences tissue behavior and function in multicellular organisms. Adult stem cells respond to nutrient availability to adjust production of differentiated cells and maintain tissue homeostasis. For example, nutrient dependent pathways act within the *Drosophila* ovary itself to regulate the number and proliferation of germline stem cells (GSCs). More recently, our laboratory showed that reduced amino acid sensing within adipocytes in the fat body also contributes to the decrease in GSC number in response to diet. The fat body is a nutrient-sensing, endocrine organ that is composed of adipocytes and hepatocyte-like oenocytes. The rise of obesity and associated chronic diseases underscores the need for understanding how adipose tissue regulates the function of other tissues within the body; however, the mechanisms whereby adipocytes influence adult stem cells in other organs in response to diet remain underexplored. To determine how adipocytes influence oogenesis in a diet-dependent manner, our laboratory previously performed a proteomic comparison to measure changes in adipocyte protein abundance when flies were switched from rich to poor diets. Strikingly, the levels of multiple extracellular matrix (ECM) proteins, including collagen type IV, are coordinately increased in the adult fat body within 12 hours of switching females rich to poor diets. Collagen type IV was previously shown to be secreted from the larval fat body and transported to other tissues through the hemolymph. Furthermore, another study demonstrated that the collagen type IV a-chain Viking (Vkg) binds to Decapentaplegic (Dpp), which is required for GSC maintenance, and that *vkg* global mutants have increased stem cell numbers in the ovary, suggesting that collagen type IV modulates Dpp signaling from the niche. We are currently testing the hypothesis that adipocyte-derived collagen type IV is transported to and incorporated in the ovary to regulate aspects of oogenesis such as GSC number.

443A

Rapid Evolution of a Germline Stem Cell Maintenance Factor. Daniel Zinshteyn, Michael McGurk, Aaron Chen, Daniel Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Germline stem cells (GSCs) are the progenitor cells for the entire population of an organism's germline. In *Drosophila*, these cells reside in a well-defined niche that is required for both their maintenance (self-renewal) and differentiation (asymmetric division resulting in a daughter cell that differs from the GSC). Dozens of genes have been implicated in each of these processes, most of them being required for production of viable gametes. The critical function of these genes suggests that they would be highly conserved across *Drosophila* taxa, but population genetic analyses have shown that many of them have undergone rapid evolution. One striking example is the stem-cell maintenance factor Stonewall (Stwl), which has experienced recurrent positive selection between *D. melanogaster* and *D. simulans*. Stwl co-localizes with HP1a and is a known suppressor of position-effect variegation; these and other results suggest that it is required for determining or maintaining a heterochromatic state. We show that ovaries mutant for *stwl* exhibit derepression of specific transposable elements. We have also discovered a novel function for *stwl* in males, finding that it is required for fertility in males as they age. We are now performing RNA-seq on *stwl* mutant ovaries and testes to assay the transcript abundance of transposable elements genome-wide in the absence of a functional Stwl. Our findings suggest that *stwl* is a target of positive selection as a response to rapidly evolving TEs. .

444B

Regeneration in the adult *Drosophila* brain. Kassi L. Crocker[1,2], Stacey Rimkus[2], Grace Boekhoff-Falk[1,2]. 1) Genetics Training Program, University of Wisconsin-Madison, Madison, WI; 2) Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI.

The regulation of neural and glial progenitors is crucial both during development and for adult homeostasis. Although *Drosophila* is a leading model for neural development, this system has not been exploited to investigate regeneration in the adult brain. This is primarily because adult brains exhibit limited mitotic activity and were thought to be unable to regenerate. Nonetheless, using both EdU labeling and pH3 staining, we observe extensive proliferation in response to penetrating head injuries of the central brain. In addition, central brain injury leads to upregulation of the pan-neuronal protein Embryonic lethal, abnormal vision (Elav) and the pan-glial protein reversed polarity (Repo). Use of the permatwin lineage tracing methods reveals that new neurons are made in response to injury, and that these newly born neurons can innervate correct target regions, suggesting that there may be functional recovery. Indeed, although climbing assays indicate significant impairment of motor function following a penetrating head injury, climbing ability improves over a period of several weeks. The discovery of cell proliferation and the generation of appropriately targeted new neurons following penetrating injury to the central brain, provides an innovative *in vivo* experimental system for further exploration of neuroregeneration.

445C

Induction of malignant neural stem cells through early evasion of temporal patterning in *Drosophila*. Cedric Maurange, Karine Narbonne-Reveau, Caroline Eple, Elodie Lanet, Sophie Foppolo. IBDM (Aix-Marseille University/CNRS), Marseille, France.

In *Drosophila*, the number of asymmetric divisions a neural stem cell (called neuroblast) can perform during development is limited by an intrinsic counting mechanism encoded by sequentially expressed "temporal" transcription factors. However, how temporal transcription factors regulate neuroblast growth and mitotic potential over time is not known. Moreover, asymmetric division defects are known to cause unlimited neuroblast amplification and malignant tumors, suggesting a disrupted counting mechanism. Yet, the underlying mechanisms causing unlimited neuroblast proliferation are still elusive. We have found that malignancy is only induced when defective asymmetric divisions are initiated in young neuroblasts during early steps of temporal factor progression. Subsequent tumors are propagated by a subset of amplified neuroblasts behaving as cancer stem cells. They indefinitely retain proliferative

properties of young neuroblasts due to the aberrant maintenance of an early oncogenic loop. This oncogenic loop involves a transcription factor and an mRNA-binding protein that are normally only expressed in young neuroblasts during early development. In normal ageing neuroblasts, the loop is silenced during late larval stages upon temporal factor progression ensuring timely termination of neurogenesis. Our study therefore reveals that temporal factor progression limits neuroblast growth and mitotic activity by silencing an oncogenic loop during late stages of development. Moreover, this work links early asymmetric division defects to aberrant temporal patterning, and the subsequent maintenance of early oncogenic loops to the induction of malignancy. It may provide a model for why pediatric neural tumors, that have an early developmental origin, often rapidly progress to malignancy in humans.

446A

Regulators of mushroom body neuroblast apoptosis. Matthew Pahl, Sarah Siegrist. Biology, University of Virginia, Charlottesville, VA.

A remarkable number of molecularly, morphologically, and functionally distinct neurons are generated during development through the asymmetric cell divisions of a diverse population of neural stem cells known as neuroblasts. Once development is completed, mushroom body neuroblasts (MB NBs), a subset of brain neuroblasts which generate neurons important for memory and learning, are eliminated by apoptosis just prior to adult eclosion. To better understand the molecular mechanism of MB NB apoptosis, we are better characterizing the role of the known *Drosophila* pro-apoptotic regulators in orchestrating this event. We have determined that *grim* and *sickle* but not *reaper* are required for MB NB apoptosis. We are working to further determine whether *grim* and *sickle* function synergistically or redundantly in this process. In addition, we have initiated an RNAi screen based on candidate genes in order to identify upstream regulators of MB NB apoptosis.

447B

The Drosophila Sp8 Transcription Factor Buttonhead Prevents Premature Differentiation of Intermediate Neural Progenitors. Yonggang Xie[1], Xiaosu Li[1], Xian Zhang[1], Shaolin Mei[1], Hongyu Li[1], Andreacarola Urso[2], **Sijun Zhu**[1]. 1) Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY; 2) Department of Biology, Syracuse University, Syracuse, NY.

Intermediate neural progenitor cells (INPs) need to avoid differentiation and cell cycle exit while maintaining restricted developmental potential, but mechanisms preventing differentiation and cell cycle exit of INPs are not well understood. Here we report that the *Drosophila* homolog of Sp8 transcription factor Buttonhead (Btd) prevents premature differentiation and cell cycle exit of INPs in *Drosophila* larval type II neuroblast (NB) lineages. We show that loss of Btd leads to elimination of mature INPs due to premature differentiation of INPs into terminally dividing ganglion mother cells. We provide evidence to demonstrate that Btd prevents the premature differentiation by suppressing the expression of the homeodomain protein Prospero in immature INPs. We further show that Btd functions cooperatively with the Ets transcription factor Pointed P1 to promote the generation of INPs. Thus, our work reveals a critical mechanism that prevents differentiation and cell cycle exit of *Drosophila* INPs.

448C

***dSox9* is required for intestinal stem cell proliferation and differentiation in the adult *Drosophila* midgut. Qing Lan**, Min Cao, Huaqi Jiang. Department of Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Understanding how adult tissue homeostasis and regeneration are regulated is one of the key questions in stem cell biology. The adult *Drosophila* midgut epithelium, which is constantly replenished by multi-potent intestinal stem cells (ISCs), serves as an excellent genetic model to investigate the underlying mechanisms. We have discovered that FoxA transcription factor *forkhead* (*fkh*) maintains the intestinal stem/progenitor cell fate, by collaborating with the *Notch* downstream E-protein transcription factor *daughterless* (*da*). To further unveil other potential adult stemness genes that are downstream of*fkh* and *da*, we performed genome-wide ChIP-seq on HA tagged *fkh* and *da* specifically induced in the stem/progenitor cells. We identified 3,188 and 5,859 binding regions for *fkh* and *da* respectively in the genome. Over half of *fkh* binding regions are shared by *da*, further suggesting their cooperative roles in determining the ISC fate. One of the genes whose promoters are co-bound by *fkh* and *da* is*Sox100B* (*dSox9*), the *Drosophila* homologue of mammalian *Sox9*. Previous studies have shown that *Sox9* is specifically expressed in the intestinal crypts (stem/progenitors and Paneth cells). Interestingly, in *Drosophila* gut epithelium we found that *dSox9* is also specifically expressed in the stem/progenitor cells. Knocking down *dSox9* suppresses the compensatory ISC proliferation in response to injury. Furthermore, we generated *dSox9* null ISC clones in the adult midgut. Although the clones grow normally, all mutant cells maintain progenitor-like small size, and lack mature cell markers for absorptive (enterocytes) and secretory (enteroendocrine cells) cell lineages, suggesting that *dSox9* is also required for ISC differentiation during homeostasis. Therefore, by genome-wide ChIP-seq profiling of the potential targets of *fkh* and *da*, we have identified and characterized another critical intestinal stem/progenitor transcription factor *dSox9*. We will report detailed genetic analysis of its function in the intestinal progenitors. In addition, we speculate that *fkh*, *da* and *dSox9* comprise of an ISC transcriptional network that determines the intestinal stem cell fate in the adult *Drosophila* midgut.

449A

Cell fate determination in intestinal stem cell progeny. Jérémy Sallé, Allison Bardin. Institut Curie, Paris, France.

In self-renewing adults tissues, newly formed undifferentiated cells are directed to different possible terminal cell fates. Thus, cell fate determination is essential for tissue homeostasis as it controls the renewal of terminally differentiated cell types and their relative proportions in the tissue. Intestinal stem cells (ISCs) in the adult *Drosophila* midgut were shown to self-renew and to produce two differentiated cell types: enterocytes (EC) and enteroendocrine cells (EE). Interestingly, the gut epithelium is composed by a vast majority

of EC while EE represent only 20% of the differentiated cells. Thus, the cell fate choice between EE and EC is critical for proper tissue homeostasis yet remains poorly understood. We first addressed the question of a non-autonomous control of cell fate decision. Using a new local EE ablation technique we found that an EE-depleted region does not increase the production of EE in the surrounding tissue suggesting that a negative feedback mechanism from EE toward ISCs is either very limited or absent. This led us to investigate potential cell autonomous control of fate choice. The mechanisms governing fate choice seem to be conserved between fly and mouse, with the Notch signaling pathway promoting absorptive fate and inhibiting endocrine fate. Importantly, mechanisms that would block Notch signaling to allow secretory specification are unknown. We have identified the negative regulator of Notch signaling, *numb,* as an essential regulator of EE cell fate: *numb* mutant tissue is defective in cell fate specification of EEs but not EC or ISC self-renewal. In addition, we find that during ISC division, Numb can be segregated either asymmetrically or symmetrically, raising the possibility that the nature of the cell division determines the fate outcome of differentiating progeny. Our ongoing work defining Numb-mediated EE cell fate choice will be discussed. The terminal cell fate choice between EE and EC provides an excellent yet simplified model in a renewing adult tissue in which to understand mechanisms of homeostatic cell fate decisions. This work provides new insight into secretory cell fate specification in the intestine.

450B

Defining tissue injury responses at the midgut/hindgut boundary with single-cell resolution. Jessica Sawyer, Emily Bowie, Ruth Montague, Donald Fox. Pharmacology & Cancer Biology, Duke University, Durham, NC.

How neighboring organs establish and maintain their boundaries during development and following tissue injury is a poorly understood phenomenon. The *Drosophila* midgut/hindgut boundary is an excellent model to study boundary integrity during development and after injury. Much of the *Drosophila* midgut is comprised of stem/progenitor cells (Escargot/Esg), enteroendocrine cells, and enterocytes (Myo1a and Pdm1). Adjacent to the midgut is the pylorus, an intestinal valve that expresses the transcription factor b*rachyenteron* (*byn*). In between the posterior midgut and pylorus is a ring of cells that are Wingless+ (Wg). Previous studies suggest that: 1) larval Byn+ cells can transdifferentiate to produce both hindgut (ectoderm) and posterior midgut (endoderm) cells, while 2) adult Wg+ cells may function as repair stem cells after tissue injury. To investigate the distinct identity and function of midgut/hindgut boundary cells, we employed a detailed marker and lineage analysis. We find that the distal pylorus strongly expresses *byn* and the cell membrane marker *fasciclin III* (*fasIII*), while the anterior pylorus expresses a low level of *byn* and *fasIII*. Further, just anterior to the *byn/fas* low cells is a ring of cells that strongly express *wg*, little to no *byn/fasIII*, and are unexpectedly Pdm1+ and Myo1a+. While this marker analysis could suggest that these Wg+ cells are enterocytes, the nuclei of these cells are much smaller than the Pdm1/Myo1a+ cells found in the anterior midgut. In addition, just anterior to the Wg ring we often find a cluster of Esg+ cells. By lineage analysis, we find that the midgut/hindgut boundary region contains distinct clone patterns not observed in the rest of the midgut or hindgut. Therefore, we have defined a distinct and novel cell intestinal boundary cell population. Upon adult hindgut damage, most of the pylorus undergoes hypertrophy. We also find the cluster of Esg+ cells adjacent to the Wg ring greatly expands in response to hindgut damage, suggesting this population of midgut cells can respond to organ damage in the neighboring hindgut. In conclusion, we have defined a novel cell population and developed a model to understand how cells at organ boundaries respond to tissue damage. .

451C

Epithelial-derived BMPs are the niche signals for *Drosophila* intestine stem cell self-renewal. Ai-Guo Tian, Jin Jiang. Department of Developmental Biology, UT Southwestern Medical Center, Dallas, TX.

In adult life, many organs rely on stem cells to maintain their integrity by replenishing lost cells during tissue homeostasis and regeneration, yet the regulatory mechanisms that control stem cell proliferation, self-renewal, and differentiation are still not fully understood. We are studying these mechanisms by using the Drosophila adult midgut as a model. Here we demonstrate that epithelia-derived BMPs serve as niche signals to promote ISC self-renewal in the midgut. We find that Decapentaplegic (Dpp) and Glass-bottom boat (Gbb) are produced by enterocytes with Dpp exhibiting basal enrichment, and that Dpp and Gbb act in conjunction to promote ISC fate by antagonizing Notch signaling. Extracellular matrix proteins at basement membrane regulate ISC self-renewal by controlling the range of BMP signaling. Furthermore, we found that epithelial damage caused by bleomycin feeding increased the production of both Dpp and Gbb in ECs as well as BMP signaling activity in precursor cells, which contributes to the increased ISC pool size. In conclusion, our data suggest that BMP signaling acts as a niche signal to regulate the ISC cell fate, and the employment of midgut epithelia as a niche for stem cell self-renewal provides a mechanism for direct communication between the stem cell niche and the environment, allowing the production of niche signal and stem cell number to be fine-tuned in response to various physiological and pathological stimuli.

452A

"Receptome-wide" RNAi screen in vivo identifies novel regulators of ISC activity in *Drosophila* midgut. Charles Xu[1], Claire Hu[1], Junjie Luo[2], Norbert Perrimon[1]. 1) Genetics Department, Harvard Medical School, Boston, MA; 2) Neurosciences Research Institute, University of California, Santa Barbara, CA.

Intestinal stem cells (ISCs) are responsible for cell replenishment in adult *Drosophila* midgut. Similar to their mammalian counterparts, these multipotent stem cells can adjust their proliferation rate in response to tissue damage and environmental stimuli, and give rise to enteroendocrine and enterocyte lineages. The essential developmental pathways regulating *Drosophila* and mammalian ISC activity are conserved. But it remains intriguing what are the upstream signals that regulate and coordinate these pathways, namely, how ISCs sense

their environment to modulate their behavior in different physiological conditions. To address this question, we have developed a luciferase reporter system to measure ISC pool size in the whold midgut organ, and performed *in vivo* genetic screen to identify receptors that are important for ISC activity. One of the interesting candidate is Transient receptor potential cation channel A1 ortholog (TrpA1). Its depletion in ISCs can inhibit their mitosis but increase differentiation. TrpA1, a calcium channel whose gating is regulated by various irritants, is responsible for nociception in the neurons. TrpA1 is expressed in the digestive tract of both *Drosophila* and mammals but its function is barely understood. We have discovered that TrpA1 is important of ISC activity, and ISCs can utilize a neuronal machinery to sense their environment.

453B

Sensory regulation of calcium signalling in hematopoiesis. Katrina Gold[1], Kalpana Makhijani[2], Brandy Alexander[1], Jenny Kim[1], Katja Brückner[1,3,4]. 1) Dept of Cell and Tissue Biology, UCSF, San Francisco, CA; 2) Dept of Pharmaceutical Chemistry, UCSF, San Francisco, CA; 3) Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA; 4) Cardiovascular Research Institute, UCSF, San Francisco, CA.

A major question in developmental biology and tissue homeostasis is how environmental stimuli regulate cell proliferation, differentiation and survival, ensuring an animal's adaptation to its environment. It has recently become clear that sensory neurons and other elements of the peripheral nervous system (PNS) are important constituents of stem cell and tissue microenvironments. Thus the PNS serves as a critical link between the external environment and local tissue microenvironments. We are using *Drosophila* larval hematopoiesis as a model to elucidate the molecular mechanisms underlying this regulatory relationship. During larval development, embryonic blood cells (hemocytes) persist and localize to specialized hematopoietic microenvironments in the body wall, known as Hematopoietic Pockets (Makhijani et al. 2011, 2012). Sensory neurons are an essential component of these hematopoietic sites, supporting the trophic survival of hemocytes, and promoting their proliferation by the production of Activinβ, a TGF-β family ligand. Experiments to manipulate PNS activity revealed that sensory neuron activation regulates the size of the larval blood cell pool and enhances Activinβ production. However this work also suggested additional molecular pathways, downstream or in parallel with PNS neuron activation. In particular, we have gained evidence that intracellular calcium modulates the hemocyte pool. Extrinsic sensory stimuli trigger increased intracellular calcium in hemocytes, as measured by GCaMP fluorescence. RNAi-mediated disruption of calcium signaling results in a decrease in total blood cell number, and perturbs hemocyte localization. Calcium signaling gain-of-function also affects larval hemocytes. Currently, we are investigating mechanisms of calcium signaling in larval hemocytes, and how calcium signaling may be influenced by PNS activity during development. .

454C

Gal4 screening for introducing new insights into *Drosophila* hematopoiesis. T. Tokusumi, Y. Tokusumi, M. Brahier, V. Lam, J. Stoller-Conrad, P. Kroeger, R. Schulz. Biological Sci, Univ Notre Dame, Notre Dame, IN.

UAS-Gal4 system is an excellent research tool to analyze various genes in *Drosophila*. Recently large-scale collections of Gal4 lines have been established, expanding the breadth of these analyses. Unlike previous enhancer trap lines, these new transgenic lines have small DNA fragments (2~3 kb) linked to Gal4 genes. This new approach has several advantages allowing researchers to view gene expression pattern in each tissue, identify regulatory regions to direct gene expression in target tissues, and use tissue-specific tools—such as a marker or Gal4 driver—to induce the expression of interesting genes in target tissues. In this study, we performed Gal4 screening in larval hematopoietic tissues: the lymph glands and the hemolymph. During larval development, the embryonic head mesoderm gives rise to hemocytes found in the hemolymph. The lymph gland is a larval hematopoietic organ and is composed of three paired lobes. In third instar larvae, the primary lobes of the lymph gland consist of three parts: the medullary zone (MZ), the cortical zone (CZ) and the posterior signaling center (PSC). The CZ is occupied by mature blood cells, while the MZ is composed of blood progenitor cells; the PSC functions as a hematopoietic stem cell-like niche. There are three mature blood cell types in Drosophila: plasmatocytes, crystal cells, and lamellocytes. Plasmtocytes are small round cells with phagocytic capacity. Crystal cells carry prophenol oxidase, which is involved in melanization. Lamellocytes are large flat adherent cells that, under normal conditions, are found in very small populations. However, under specific conditions such as wasp parasitization, numerous lamellocytes are induced and encapsulate foreign enemies. We crossed approximately 1000 lines of Fly Light Project Gal4 lines with UAS-GFP lines and observed expression patterns in both the lymph glands and the hemolymph, classifying the hemocyte types and the parts of the lymph glands. We also analyzed these enhancer/regulatory regions of these Gal4 lines and associated genes and will report these results. .

455A

Differential levels of PI3K/TOR signalling determine the outcome of competition between stem cells for differentiation. Marc Amoyel[1,2], Kenzo-Hugo Hillion[1], Shally Margolis[1], Erika Bach[1,2]. 1) Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA; 2) The Helen L. and Martin S. Kimmel Center for Stem Cell Biology, New York University School of Medicine, New York, NY, USA.

While stem cell self-renewal has been extensively studied, it is generally presumed that differentiation is a default pathway that occurs upon removal of self-renewal factors. Stem cell niches provide the requisite signals for self-renewal. Upon division, a cell that is pushed out of the niche is no longer exposed to this permissive environment and initiates differentiation. However we show that differentiation is an active process that requires signalling in the PI3K/Tor pathway in somatic stem cells (CySCs) in the *Drosophila* testis. We show that differentiating cells display elevated PI3K activity and that knock down of the PI3K effector Akt1 prevents normal CySC differentiation, suggesting that elevated metabolic activity is required during differentiation. Finally, although we previously showed that clones with

elevated PI3K or Tor activity differentiate, hyper-activation of PI3K throughout the somatic lineage does not lead to forced differentiation. These results suggest that stem cells are normally competing with each other to differentiate and that the outcome of this competition is determined by the relative levels of PI3K/mTor activity between somatic cells. Thus we establish enhanced metabolism as a central regulator of differentiation.

456B

Quiescent cells in the adult Drosophila testis niche are not replenished after damage. P. Hétié, **M. de Cuevas**, E. Matunis. Department of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

In the adult Drosophila testis, germline stem cells (GSCs) and somatic cyst stem cells (CySCs) adhere to a cluster of post-mitotic cells called the hub, which promotes GSC and CySC maintenance by local secretion of the JAK-STAT pathway ligand Unpaired. Although hub cells were considered to be terminally differentiated, we recently showed that they are more plastic than once thought: genetic ablation of CySCs, or overexpression of Cyclin D (CycD) and Cyclin dependent kinase 4 (Cdk4) in the hub, causes hub cells to exit quiescence, delaminate from the hub, and convert into functional CySCs (Hétié et al, Cell Reports, 2014). Here, we ask if the testis has a similar mechanism to replace lost or damaged hub cells. We developed methods to genetically ablate hub cells in the adult testis and found that flies recovering from hub cell ablation, in contrast to CySC ablation, do not replace missing hub cells. Furthermore, when we labeled dividing cells before or after ablation of hub cells, we did not find labeled cells in the hub at any time point. We conclude that hub cells do not exit quiescence in response to ablation and that proliferating cells do not enter the hub before or after ablation. Taken together, our results suggest that the adult testis niche does not have a mechanism to restore lost or damaged hub cells..

457C

Investigating a Role for JAK/STAT Cytokine Signaling in Mushroom Body Neuroblast Apoptosis. Xin Yuan, Sarah Siegrist. Biology, University of Virginia, Charlottesville, VA.

Mushroom body neuroblasts (MB NBs) generate neurons important for memory and learning, and persist late into pupal stages of development. Prior to eclosion, MB NBs are eliminated by programmed cell death. It remains unclear whether extrinsic factors, either local or systemic, control timing of MB NB cell death. We are investigating whether MB NBs like other stem cells may reside within a unique microenvironment, or niche, that provides trophic support and shields stem cells from deleterious growth factors or other cytokines in the extracellular environment. Because little is known about either the cellular or molecular composition of the NB stem cell niche, we are exploring candidate stem cell niche regulators from other systems. Here we show that during development, MB NBs and their recently born progeny are ensheathed by glial cells that have elevated levels of JAK/STAT cytokine signaling activity. In addition, we show that Unpaired (Upd), the ligand for JAK/STAT activation, is expressed in neurons generated by the MB NBs themselves. Could MB neurons act like the "hub" from the male germline to regulate JAK/STAT signaling in glial cells and support MB NB proliferation.? We propose that ensheathing glia and MB neurons are two cell types that may constitute the MB NB niche and are further investigating a role for JAK/STAT activity in regulation MB NB apoptosis. .

458A

Ecdysteroids and miRNA cooperate in regulating differentiation of germline stem cell progeny. Annekatrin König, Andriy S Yatsenko, Halyna R Shcherbata. Research Group of Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Since egg production is a highly energy demanding process, it has to be tightly regulated. While a great deal is known about the mechanisms controlling adult stem cell behaviour in the niche, it is not clear whether differentiation of the stem cell progeny is the default program because stemness could not be maintained away from the niche, or whether additional signaling inducing differentiation is needed. Previously, we showed that ecdysteroid signaling is critical for early germline differentiation: ecdysone signaling dysfunction causes germline stem cell progeny to delay the differentiation program. The delayed germline state is characterized by the specific pattern of the germline markers and particular chromatin modifications. Monoubiquitination of histone H2B (that is present in differentiating germline cysts) is absent from the germline cells, delayed upon ecdysone signaling perturbation. We found ecdysone signaling to be mainly active in the somatic cells, thus, the differentiation delay observed in the germline is a result of cell non-autonomous steroid regulation. Interestingly, the miRNA *let-7* that targets Abrupt, a repressor of ecdysone signaling in the germarial soma, modulates the tissue-specific response to this hormonal signaling. Upon stress or aging the levels of*let-7* change. Furthermore, *let-7* in the adult ovary is itself controlled by ecdysteroid signaling. Therefore, *let-7* acts in a feedback loop to fine-tune ecdysone signaling intensity in accordance with internal and external cues. Intriguingly, *let-7* is differentially expressed in testes and ovaries. Loss of *let-7* or ecdysone signaling perturbation both affect gonadal soma shape and behaviour, leading to delayed germline differentiation in both sexes. In addition, sex determination genes normally restricted to the opposite sex are misexpressed upon steroid (or *let-7*) deficit. Thus, *let-7* thus is a mediator of ecdysteroids that maintain sexual identity in male and female gonads.

459B

Drosophila crystal cells undergo pyroptosis to release pro-phenoloxidase at wound sites. Robert Krautz[1], Zhi Wang[1], Robert Markus[2], Ulrich Theopold[1]. 1) Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Stockholm, Sweden; 2) University of Nottingham School of Life Sciences E100, E Floor, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH.

Immune systems of all living organisms face the challenge to cope with suddenly occurring threats, ranging from inflicted wound

sites, the infiltration of pathogens or internal tissue damage. Potent innate immune responses evolved as the first line of defense against those instant incidents in order to contain the immune challenge on a local scale and thus prevent their systemic spreading, which would otherwise lead to autoimmune responses and jeopardize tissue homeostasis in the surrounding. Both, the necessity for an immediate and intense immune response and the need to focus these responses locally demand for tight regulation of the deployed effector mechanisms. The melanisation reaction in Drosophila melanogaster and its activation serves as a prototypical example for those demands. Here, we show that crystal cells which deliver the key enzyme for the melanisation cascade, pro-phenoloxidase (PPO), undergo a highly regulated mode of programmed cell death that bares striking resemblance to pyroptotic cell lysis described for the activation of immune cells in vertebrate immune systems. Apart from cellular characteristics such as the restructuring of the cell cortex, swelling and rounding prior to rupture of the plasma membrane, we confirm a dependence on caspase activation throughout the process. All of those features were previously identified as hallmarks of the pyroptotic death program. Not only does the rupture of crystal cells exhibit a potent immune response, but its activation via caspases allows for tight regulation of its exertion as shown for the diminished melanisation in wounds. Hence, crystal cell rupture provides a suitable model for studying pyroptosis and will due to the possibilities of genetic modifications in Drosophila hopefully reveal further insights into the molecular sequence of events prior to membrane rupture.

460C

Belle is functionally required for the expression of transgenes and subsets of transposable elements. Pang-Kuo Lo, Yi-Chun Huang, William Palmer, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

Belle (Bel), the *Drosophila* homolog of the yeast DEAD-box RNA helicase Ded1p and human DDX3, has been shown to be required for *Drosophila* oogenesis and female fertility. However, the biological function of Bel remains largely unknown. Here we have revealed a novel role of Bel in regulating the expression of foreign, invading DNA such as transgenes and transposable elements. Abrogation of Bel by mutations induces silencing of a variety of transgenes. This silencing effect is attributable to the downregulation of their RNA levels. Our studies have further shown that the RNA helicase Spindle-E and the chromatin remodeling factor Mod/Mdg4 are both required for Bel-related transgene silencing as inactivation of either one fully or partially rescues the *bel* mutant phenotype. Spindle-E plays a crucial role in PIWI-interacting RNA (piRNA) biogenesis, a cell immune mechanism to silence expression of transposable elements for maintaining genomic stability. To explore whether Bel is also involved in piRNA biogenesis, we performed deep sequencing analysis, which revealed that Bel has no direct role in regulating canonical piRNA biogenesis from the heterochromatic piRNA cluster loci. Nevertheless, Bel is found to be required for the expression of subsets of transposable elements via an unknown mechanism. Furthermore, we found that inactivation of Bel could rescue the*Spindle-E* mutant phenotype manifesting mislocalization of nuage protein components. This suggests that Bel and Spindle-E have a competitive relationship in the nuage of germline nurse cells, a subcellular site for multiple RNA processing including piRNAs. Our findings provide further insight into the functional role of Bel in the cell immune system and its implications towards human DDX3-related tumorigenesis, and for the first time genetically link Bel to the two protein regulators, Spindle-E and Mod/Mdg4, involved in RNA processing and chromatin remodeling, respectively.

461A

Role of a lysosomal chloride transporter in the phagocytic degradation of bacteria. Ching-On Wong, Hongxiang Hu, Yufang Chao, Meera Namireddy, Kartik Venkatachalam. Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX.

Phagolysosomal degradation depends on the ionic homeostasis within those vesicles. Therefore, mutations in genes responsible for the movement of ions across phagolysosomal membranes may lead to diminished degradation of internalized cargo. Here, we report our findings with *Drosophila* lacking a lysosomal chloride/proton antiporter, Clc-b, which is the homolog of mammalian Clcn7. Loss-of-function mutations in mammalian *clcn7* lead to osteopetrosis due to dysfunctional osteoclasts—a cell type of hematopoietic origin. Although flies don't have monocytes, the macrophage equivalent cell-type, hemocytes, are also hematopoietic in origin. Therefore, we examined hemocyte function in flies lacking *clc-b* (*clc-b*^{1}) using a bacterial-clearance assay. The *clc-b*^{1} mutants showed defective bacteria clearance compared to controls. This phenotype was suppressed by hemocyte-specific, but not fat body-specific, expression of *clc-b* cDNA, indicating diminished cellular immune responses in *clc-b*^{1}. Further analysis revealed that although naïve *clc-b*^{1} hemocytes that were not previously exposed to *E. coli* showed no change in acute phagocytosis of the bacteria, the ingested bacteria were not degraded in these hemocytes even 2-days after uptake. Our findings demonstrate the importance of lysosomal chloride homeostasis in the phagocytic degradation of bacteria. Remarkably, diminished degradation of bacteria in *clc-b*^{1} led to a transcriptional downregulation of the phagocytosis receptor, *eater*. As a consequence, *clc-b*^{1} hemocytes were unable to maintain their ability to internalize bacteria resulting in a compromised cellular immune response over time. In summary, our findings suggest that bacterial degradation is required for the priming of a transcriptional program necessary for the continued phagocytic uptake of bacteria. .

462B

Dual role of *Beadex* in Drosophila immunity. Arunita Chatterjee, Kumar Aavula, Esha Patnaik, Upendra Nongthomba. Dept of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India.

Drosophila melanogaster mounts a potent immune response against pathogens that cross its physical barrier. *Drosophila* has two arms of immunity – Humoral and Cellular. While humoral immune system works by synthesizing Anti-Microbial Peptides (AMPs), cellular immune response is mediated through phagocytosis by plasmatocytes and melanization by crystal cells. The development and function of Drosophila immune system is fairly well-studied. However, several missing links and ambiguities make the study of novel genes

implicated in Drosophila immunity necessary. This study attempts to elucidate the role of *Beadex* (*Bx*) in Drosophila immunity. *Bx* codes for a LIM-only protein and has been implicated in Drosophila immune response in two independent screens. We observed that mutants of *Bx* were more susceptible to infection and unable to synthesize AMPs. Knocking down *Bx* in fat-body, the primary producer of AMPs, drastically reduced survival upon infection. Further, through molecular studies, we show that *Bx* affects AMP production by regulating the expression of DREDD, a caspase required for activating the Imd pathway. While assessing the role of *Bx* in cellular immune response, we found alterations in the hemocyte counts in *Bx* hypermorphs. Total hemocyte numbers were reduced, whereas crystal cell counts were significantly higher in *Bx* hypermorphs. *Bx* over-expression in pro-hemocytes altered the development of crystal cells, similar to *Bx* hypermorphs. The pool of hemocytes specified towards crystal cell lineage is regulated by *ushaped* (*ush*) and *serpent* (*srp*, a GATA factor) complex. In this study, using mis-expression approach, we establish the role of *pannier* (*pnr*, a GATA factor) in the regulation of crystal cell progenitor population through its physical interaction with ush. Further, through genetic studies, we show that *Bx* is epistatic to *pnr*. We thus establish the mechanism of two novel regulators of crystal cell specification during Drosophila hematopoiesis.

463C

The microbiota induces Pvf2 to activate the antiviral ERK pathway in the Drosophila gut. Jonathan Cohen, Christine Sansone, Ari Yasunaga, Jie Xu, Beth Gordesky-Gold, Sara Cherry. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Various pathogens, including viruses, can be orally acquired and thus infect cells within the gastrointestinal tract. Arthropod-borne viruses (arboviruses) are a globally important class of pathogens transmitted to humans via blood feeding arthropods, which become infected orally during feeding. We set out to discover molecular mechanisms that control antiviral immunity in the gut using the genetically tractable model Drosophila Melanogaster. We previously found that the conserved ERK signaling pathway controls innate immunity within the intestinal epithelium: flies mutant for this pathway display increased susceptibility to a panel of viral pathogens. The ERK pathway is canonically activated by Receptor Tyrosine Kinases, and through RNAi screening we discovered that PVR (PDGF/VGF Receptor) is required for antiviral ERK signaling in the intestinal epithelium. Furthermore, we found that the PVR ligand Pvf2 is both induced by viral infection and required for antiviral defense in the intestine. Moreover, we found that the microbiota plays a role in this response. The microbiota is required for virus-induced Pvf2 induction and thus controls antiviral responsiveness. Ablation of the microbiota leads to increased viral infection and a lack of antiviral ERK signaling. Altogether, these findings extend our understanding of local antiviral defenses. Elucidating the mechanisms by which insects respond and restrict arbovirus infection will potentially aid in our development of antiviral strategies to intervene in the insect vector and block transmission of this important class of pathogens.

464A

***zfh2* is an *in vivo* mediator of hypercapnic immune suppression. Ryan Haake**, James Kwon, Greg Beitel. Dept. of Molecular Biosciences, Northwestern University, Evanston, IL.

Obstructive pulmonary diseases, including chronic obstructive pulmonary disease (COPD) and Cystic Fibrosis, are the fourth leading cause of death in the United States. Patients with these diseases often have elevated CO_2 levels (hypercapnia), which is associated with increased pulmonary infections and death. Previously, our lab used an S2 cell-based RNAi screen to identify candidate genes that modulate immune suppression by elevated CO_2 levels. Knock down of candidate genes reversed the suppression of Diptericin by CO_2 and restored expression in elevated CO_2 towards levels observed in air. We are currently testing candidate genes to determine if *in vivo* knockdown abrogates increased mortality of bacterially infected flies in elevated CO_2. One candidate gene whose knockdown reduces the effects of hypercapnia both *in vitro* and *in vivo* is *zfh2*. Whereas a 48 hour pre-treatment in 13% CO_2 significantly increased mortality ofw^{1118} infected with *S. aureus*, $zfh2^{209}$ mutants do not. $zfh2^{209}$ reduces the effect of hypercapnia on resistance rather than tolerance to bacterial infection since hypercapnia increases bacterial load inw^{1118} but not $zfh2^{209}$ mutants. To identify potential tissues where *zfh2* may be acting, we knocked down *zfh2* in the fat body using CG-Gal4 and the $zfh2^{13305}$ UAS-RNAi line. As with$zfh2^{209}$ homozygotes, neither increased mortality nor bacterial load was observed in flies in which *zfh2* had been knocked down in the fat body. For these experiments, we used a new approach to determine bacterial load. Sixteen hours after inoculation, individual flies were homogenized in LB, and the supernatant inoculated 2 ml of LB, followed by incubation and measurement of optical density. The results are comparable to CFUs determined by plating serial dilutions; however the liquid culture technique is faster and more cost-effective than traditional CFUs. Preliminary experiments using the muscle-specific dMef-Gal4 indicate that knockdown of *zfh2* in muscles is not protective against hypercapnic induced immune suppression. Together these results identify *zfh2* as a mediator of hypercapnic immune suppression and identify the fat body as an important site of action of hypercapnic immune suppression.

465B

A chromatin remodeling factor contributes to innate immune homeostasis in intestine. Xiaomeng He, Lei Pan. Institute of Biophysics,CAS, Beijing, China.

Metazoan lives in an environment which is full of a diverse array of microorganisms. Continuous interactions between host and microbes promote the host to evolve elaborate defense mechanisms to protect themselves. As the largest and typical mucosal surface, intestine not only acquires the most opportunities to access to the ingested microbial pathogens, but also provides habitat for commensal bacteria. Thus to its essence, the intestinal mucosa has to evolve the ability to keep the balance. By a set of genetic screening in *Drosophila*, we identified a chromatin remodeling gene *X*, which deletion caused higher morbidity and mortality than wt upon gram-negative bacteria *Ewinia carotovora 15* challenges, implicating *X* gene might play a protective role in regulating the host

innate defense against enteric bacteria. Detailed analysis revealed that *X* mutant flies had rather than an accretion of bacteria load but a boost induction of antimicrobial peptides (AMPs), inflammatory cytokine *eiger*, and Eiger mediated tissue damage which is mainly responsible for mortality during infection. Gene *X* is therefore characterized with the capability to inhibit detrimental inflammation storm. Mechanistically, in the nucleus, X could be recruited to *AMP*'s promoters by the transcriptional factor Relish of Imd signaling in order to impede the transcriptional function of Relish. Furthermore, X could also occupy the *eiger*'s promoter for the suppressive function. On the other hand, because X expression was upregulated in response to infection depending on Relish activation in the gut, it suggested that gene *X* functions as a feedback repressor of intestinal Imd signaling to bacterial infection. Moreover, flies with deficiency of X associated with chronic inflammatory responses and shorten lifespan in the present of commensal flora, indicating the participation of gene *X* in regulating intestinal immune homeostasis intrinsically. Taking advantage of knockout mice, we found that gene X played conserved function in mammalian. In conclusion, our study demonstrated a novel regulatory way to keep innate immune homeostasis in the gut and even to enlighten the studies for human intestinal innate immunity and pathology.

466C

Molecular mechanisms of neuroinflammatory response in a *Drosophila* Parkinson's model. Anna Moyer, Janis O'Donnell. Biological Sciences, University of Alabama, Tuscaloosa, AL.

Although the etiology of Parkinson's disease (PD) is complex, genetic background may modulate susceptibility to environmental risks for disease development. In a *Drosophila* PD model, ingestion of the herbicide paraquat promotes oxidative stress and elicits dopaminergic neurodegeneration and movement disorders. Genetic modification of the dopamine synthesis pathway alters sensitivity to paraquat insult, while oxidative stress in a genetic model of PD induces expression of the enzyme nitric oxide synthase. Because nitric oxide synthase is involved in the immune response, we examined the mechanism of the induction and activation of the immune response in regulating sensitivity to paraquat. We found that genetic perturbation of dopamine synthesis results in alterations in response to paraquat that are mediated by differential nitric oxide synthase expression.

467A

Investigating host-microbe interactions in Drosophila. Meera Namireddy[1], Ching-On Wong[2], Yufang Chao[2], Hongxiang Hu[2], Kartik Venkatachalam[2]. 1) Rice University, Houston, TX; 2) The University of Texas Health Sciences Center-Houston, Houston, TX.

In the context of host-microbe interactions, tolerance is a poorly understood condition whereby the capacity of the host to mount an adequate immune response against the microbe diminishes over time, allowing the microbe to continue to survive within the host. To better understand host-microbe interactions in *Drosophila*, we study the "primed innate-immune response". Priming involves injecting flies with an agent such as heat-killed bacteria, which activates phagocytes such that when live bacteria are subsequently injected into these flies, the animals are able to more readily clear the live bacteria. Thus far, we have determined that in wild-type flies, the effect of priming on bacterial clearance is dose-dependent. Specifically, priming with moderate doses of heat-killed *E. coli* correlates with high levels of subsequent clearance of live bacteria. In contrast, priming with low doses of dead bacteria correlates with low levels of bacterial clearance. Interestingly, there is a threshold for this response, and if primed with an unusually high dose of dead bacteria, subsequent clearance of live bacteria is compromised, so much so that observed levels are comparable to bacterial clearance levels observed when flies are primed with low doses of heat-killed *E. coli*. Therefore, our data show that the efficacy of the priming response exhibits a bell-shaped dependence on the dose of the priming agent. We hypothesize that extremely high doses of heat-killed *E. coli* somehow disrupt the phagocytic clearance of live bacteria. Furthermore, we hypothesize that the inability of macrophages to optimally clear live bacteria after being subjected to a very high dose of priming agent may be a potential mechanism underlying the development of tolerance in *Drosophila*. Therefore, our studies may be relevant to understanding the mechanistic basis for the development of tolerance during host-microbe interactions.

468B

Post-mating reduction of immune defense in *Drosophila melanogaster* females: testing the hormonal pleiotropy hypothesis. Robin Schwenke, Brian Lazzaro. Cornell University, Ithaca, NY.

Both reproduction and immune defense are physiologically demanding processes and are important to organismal fitness. Although immune function is critical for survival, accumulating evidence suggests reproductive efforts impinge on immune defense. Despite these observations, the underlying mechanism remains unknown. We hypothesize that Juvenile Hormone (JH), which is induced by the receipt of Sex Peptide during mating, acts either directly or indirectly to suppress immunity in *Drosophila melanogaster* females. In previous work, we found that *D. melanogaster* females become more susceptible to bacterial pathogens shortly after mating. Using transgenics and genetic mutants, we identified that Sex Peptide, an important male seminal fluid protein, is required for the post-mating increase in susceptibility to *Providencia rettgeri,* a Gram-negative bacterium. Previous findings show that the N-terminus of Sex Peptide is required for the production of JH, an endocrine signalling hormone that promotes oogenesis. We find that the N-terminus of Sex Peptide is required for the effect of mating on immunity. Through ectopic application of a JH analogue, we can suppress antimicrobial peptide expression, thus reducing resistance to an infection. Similarly, the removal of JH via the genetic ablation of the corpus allatum, the JH-producing organ, rescues virgin-levels of immunity in mated females. Ongoing work seeks to identify the JH receptor involved in mediating immune suppression in mated females. .

469C

Regulatory Roles of Bromodomain Containing Proteins (BCPs) and Jumonji Domain Containing Proteins (JDCPs) in Immunity and Inflammation. J. Sharrock, M.S. Dionne. Centre of Molecular and Cellular Biology of Inflammation (CMCBI), Kings College London.

Bromodomain containing proteins (BCPs) and jumonji domain containing proteins (JDCPs) play important roles in transcriptional regulation and chromatin remodelling. The inhibition of BCPs or jumonji domain containing protein 3 (JMJD3) has been shown to affect the production of pro-inflammatory cytokines by macrophages; phagocytic cells that reside in various tissues, suggesting they may play an important role in the regulation of immunity and inflammation. We aim to identify how tissue specific knockdown of BCPs and JDCPs in *Drosophila melanogaster* affects survival following infection and whether it alters the production of antimicrobial peptides (AMPs). We use RNAi to knockdown each of the 21 identified genes encoding BCPs and JDCPs in the *D. melanogaster* genome in relevant immune tissues, including the fat body and hemocytes. Detailed analysis will focus on the immune response following bacterial infections, cytokine production and by assaying metabolic changes. The RNAi survival screen using gram-positive and gram-negative intracellular bacteria, *Listeria monocytogenes* and *Francisella novicida* respectively, identified one particular gene of interest, known as Female sterile (1) homeotic (Fs(1)h). The Fs(1)h gene is a BCP critical for ultrabithorax (Ubx) activation and plays an important role in the activation of HOX genes. Interestingly, using the RNAi specifically to knock down Fs(1)h in the fat body not only shows a reduction in survival following infection but also a number of metabolic changes. However, there is no change in survival when the RNAi is driven within the hemocytes. Until now the role of Fs(1)h within the immune response has not been investigated, and these data suggest Fs(1)h may play an immuno-metabolic role within *D. melanogaster*. .

470A

The physiological genetic basis of immune-energetic interactions in *Drosophila*. Justin Buchanan, Colin Meiklejohn, Kristi Montooth. Biological Science, University of NE - Lincoln, Lincoln, NE.

To investigate the role of energetics and autophagy in mediating immune function and trade-offs between immune function and life-history traits, such as fecundity, a set of mitochondrial-nuclear hybrid genotypes between *D. melanogaster* and *D. simulans* was used. As the result of an incompatible interaction between polymorphisms in the mt-tRNA^{Tyr} and its nuclear-encoded mitochondrial tyrosyl-tRNA synthetase *Aatm*, one of these genotypes, *(simw^{501});OreR*, has compromised oxidative phosphorylation activity, which is essential for ATP production. This genotype causes decreased female fecundity, delayed development time, and patterns of disrupted larval metabolic rate that are indicative of inefficient energy metabolism. Here we show that this genotype is also resistant to the effects of rapamycin, a drug that manipulates TOR signaling, activates autophagy and extends development time. We will present data testing the predictions that *(simw^{501});OreR Drosophila* larvae (1) intrinsically compensate for their inefficient energy metabolism by activating autophagy, explaining why they may be resistant to additional induction of autophagy via rapamycin, and (2) mount less effective immune responses in response to bacterial infection due to their inherent energy limitation. These experiments also characterize the dynamics of *Listeria monocytogenes* infection and clearance during larval development. This system is being developed as a model for studying immune-energetic interactions in *Drosophila*.

471B

Determinants of paralytic behavior after viral infection. Jonathan Chow, Jonathan Kagan. Harvard University and Boston Children's Hospital, Boston, MA.

While knowledge of the molecular interplay between host and virus has increased, the consequences of viral infection on host physiology are less understood. A disconnect currently exists between the responses observed at a molecular level and the consequences of infection observed at the organismal scale. This disconnect is most evident in the case of rhabdovirus infection of *Drosophila*. Using the rhabdovirus vesicular stomatitis virus (VSV), we have observed that septic infection and CO_2-induced narcosis in tandem cause paralytic behavior. The molecular events causing paralysis are unclear. Compared to infection with an unrelated alphavirus, Sindbis virus, we find that paralysis following CO_2 exposure is specific to VSV infection. The severity of infection can be modulated by changing the incubation temperature or by inhibiting the antiviral siRNA response. Consequently, the onset of CO_2 sensitivity begins earlier or later. We hypothesized that the variable lag time between infection and sensitivity to CO_2 depends on VSV dissemination to a specific tissue. Using cell-type specific Gal4 drivers, we restored the siRNA pathway in hemocytes, neurons, or muscles to determine which infected tissue is responsible for CO_2 sensitivity. We found that VSV mainly infects muscles but infection of neurons potentiates death after CO_2 exposure. The paralysis and death observed is specific to CO_2 since anesthetization by nitrogen gas does not result in coordinative defects. Ongoing work seeks to identify the genetic pathways responsible for CO_2 sensitivity and to understand how VSV infection modulates these pathways. By understanding how paralysis and death are triggered, we hope to develop novel methods of selectively controlling insect populations that pose a burden to human health.

472C

Production of Nora virus ORF1 monospecific antisera. Tad Fuchs, Kirsten Lipps, Brad Ericson, Darby Carlson, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Pircornaviruses are positive, single-stranded RNA viruses that have a single open reading frame, ORF, allowing for easy replication. Nora virus, a *Drosophila* picorna-like virus, is functionally like other picornaviruses, but not structurally as it has four open reading frames instead of just one. There is not much known about the individual open reading frames encoded by the virus mainly the ORF of interest,*ORF1*. It is believed to have a role in RNA interference, RNAi, suppression through inhibition of the RNA induced silencing complex, RISC. This allows Nora virus to remain persistent in its host. The purpose of this study is to produce *monospecific* antisera

specific to the *ORF1* encoded protein, viral protein 1, VP1, with validation of specificity via Western blot analysis. *ORF1* nucleic acid was codon optimized, amplified via PCR, and cloned into *pET28a*. The *pET28a-ORF1* was transformed and expressed in *BL21 E. coli* competent cells. The resulting VP1 protein was purified via affinity column chromatography. The purified VP1 protein was injected into mice for the production of monospecific antisera and collected via retro-orbital bleeding. Expression of VP1 was assessed using Western blot analysis and a product was present at approximately 60 kDa, which was the expected result. This indicated the specificity of the antisera to the VP1 antigen. Currently, this antisera is being tested for its usefulness in immunohistochemical studies to determine the site of synthesis of VP1. *In toto*, the monospecific antisera produced will be useful for the characterization of ORF1's role in Nora virus replication. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

473A

Dissecting the mechanisms by which cAMP-producing toxins disrupt junctional trafficking. Annabel Guichard[1], Stephen Chin[1], Beatriz Cruz Moreno[1], Janet Liu[2], Lin Zhu[1], Berenice Aguilar[2], Victor Nizet[2,3], Ethan Bier[1]. 1) Dept of Biology, Univ. California, San Diego, La Jolla, CA; 2) Dept of Pediatrics, Univ. California, San Diego, La Jolla, CA; 3) Skaggs School of Pharmacy , Univ. California, San Diego, La Jolla, CA.

Bacterial pathogens express toxins that disrupt key defense processes to escape immune response and disseminate within and among host organisms. Interestingly, several unrelated toxins, through distinct mechanisms, lead to excessive production of cAMP, suggesting that elevated levels of this common second messenger contribute to pathogenesis. For example, Cholera toxin (Ctx) from *Vibrio cholerae,*ADP-ribosylates the α subunit of trimeric G proteins, leading to constitutive activation of endogenous adenylate cyclases (AC), while edema factor (EF) from *Bacillus anthracis* is itself a potent AC. Using transgenic flies expressing EF or Ctx, we have uncovered a connection between cAMP overload and inhibition of Rab11, a small Rab GTPase critical for endocytic recycling of cargo proteins such as Notch ligands and cadherins to adherens junctions. This inhibition weakens intercellular junctions, leading to edema through the vascular endothelium in differing tissues (EF), or diarrhea due to fluid secretion across epithelial cells of the small intestine (Ctx). Our current analysis focuses on how cAMP causes disruption of junctional transport. Genetic epistatic experiments and examination of the sub-cellular distribution of wt and mutant candidate pathway components suggest a mechanism involving predicted cAMP mediators (PKA and Epac/Rap1), as well new players (Rip11/Arf6) that contribute to cAMP pathology. Our study is validated by experiments in human cells and mouse tissues relevant to anthrax and cholera didease pathogenesis. In a parallel effort, we are screening for compounds able to ameliorate junctional weakening induced by both toxins, and potentially mitigate the symptoms characteristic of these and possibly other pathologies.

474B

The genetic architecture of defense as tolerance and resistance. Virginia Howick, Brian Lazzaro. Entomology, Cornell University, Ithaca, NY.

Defense against pathogenic infection can take on two forms, resistance and tolerance. Resistance is the ability of a host to limit a pathogen burden whereas tolerance is the ability to limit the negative impact of that burden. Although the ecological and evolutionary consequences of tolerance and resistance are well studied, no QTL for tolerance have been identified, suggesting these traits have distinct genetic architectures. Using the *Drosophila* Genetic Reference Panel, we have performed genome-wide association studies to identify the genetic basis of natural variation in tolerance and resistance to the Gram-negative natural pathogen, *Providencia rettgeri*. We found that resistance to infection was predicted by SNPs in several humoral immune genes involved in both the Toll and IMD pathways. Tolerance was predicted by SNPs in genes involved in regulation of the cellular immune system and notch signaling. Using multivariate analyses, we have identified SNPs that are pleiotropic for resistance and tolerance, confirming these traits are intimately linked. Reanalysis of published transcriptomic data revealed that genes contributing to variation in resistance tend to be up-regulated after infection, but genes contributing to tolerance are not transcriptionally altered by infection. This observation implies that there is no induction of tolerance upon infection, but instead a predetermined state that results in tolerance; this suggests that costs of tolerance are likely associated with maintenance, whereas costs of resistance are more likely to be associated with deployment. .

475C

Production of Nora virus ORF2 monospecific antisera. Alexis Page, Brad Ericson, Darby Carlson, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a picorna-like virus that has four open reading frames (ORFs). The presence of four open reading frames is in contrast with other members of this virus family, which only contain one long open reading frame. Not much is known about the coding potentials of the four ORFs, especially ORF2. However, it is known that ORF2 is the largest ORF, and it is believed that ORF2 encodes the replicative proteins such as the RNA polymerase (RNAP) and helicase. In addition, it is predicted to specify the viral protease, which is needed to process the predicted polyproteins encoded by ORF 2 and ORF 4. The RNAP is of intense interest because it is allows the virus to replicate its RNA. The purpose of this study was to express and purify the RNAP region of ORF2, and to produce monospecific antisera in mice against this protein. The resulting ORF2 RNAP was expressed, purified, and analyzed using SDS-PAGE. The results showed a protein product at ~30 KDa, which is the expected size for ORF2 RNAP. After batch expression and purification of ORF2 RNAP, it was injected into mice and blood serum containing the monospecific antibodies was collected. The resulting monospecific antiserum was validated with Western Blot using infected *Drosophila melanogaster* lysates. The production and validation of the

monospecific antisera is a useful tool for characterizing the structure and function of ORF2 RNAP from Nora virus. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

476A

***Drosophila melanogaster* Nora virus virus-like particles: *in vitro* assembly. Ryan Sowle**, Kellie Licking-Murray, Brad Ericson, Darby Carlson, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a recently discovered RNA picorna-like virus that produces a persistent infection in *Drosophila melanogaster*. This virus is of interest because it is similar to the human picornaviruses that are responsible for human diseases, such as polio, hepatitis A, foot and mouth disease, and the common cold. Virus-like particles (VLPs) are non-infectious virions, which contain only empty capsids with no enclosed packaged genetic material. Assembly of VLPs, for diseases caused by known viruses that lack efficient treatment and prevention, could be essential in the production of vaccines. For this study, VLPs of the Nora virus ORF 1, -3, -4a, -4b, and -4c proteins were placed together, *in vitro*, to determine the gene(s) that are essential in assembling the structural capsid. Proteins were separated through cesium chloride gradients and detected by Western blot analysis. Further visual composition and aggregation of protein was confirmed by electron microscopy. Electron microscopy revealed a size distribution similar to that of wild type virus when viral protein 4A is present with an additional protein, but the lack of VP4A, or VP4A alone, results in scattered size distribution, potentially indicating VP4A as the scaffold protein. The Nora virus assembly pathway is not yet known, but consideration of this could lead to a deeper understanding of how picornaviruses in general undergo assembly. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

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477B

The POU/Oct transcription factor *nubbin (nub)* is necessary for a beneficial gut microbiota and for normal adult life-span of *Drosophila*. Ylva Engström[1], Widad Dantoft[1], Daniel Lundin[2]. 1) Dept. of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, SE-10691 Stockholm, Sweden; 2) Bioinformatics Infrastructure for Life Sciences, Science for Life Laboratory, Box 1031, SE-171 21 Solna, Sweden.

Gut homeostasis is based on the presence of a healthy gut microbiota, a well-tuned immune response and tightly controlled gut epithelium regeneration. Infections with pathogens disrupt this homeostasis leading to activation of inflammatory processes, changes in microbial community and enhanced stem cell proliferation. It is not well understood how the commensal flora is kept steady, so that it is neither eradicated, nor allowed to overgrow and invade the organism. We have previously shown that in nub^{1} mutants, the immune system is constantly active due to transcriptional de-repression. Here we analyzed how this aberrant immune gene activity affected the gut microbiota and fly health, by comparing the microbial composition, immune gene activation and survival curves of nub^{1} compared to *OrR* flies. By using Illumina-based 16S rRNA gene sequencing we found that the taxonomic composition of the gut microbial community of laboratory-reared flies was much larger than previously reported. Surprisingly,nub^{1} mutant flies had higher loads of bacteria and a more diverse taxonomic composition than *OrR*. In addition, the life-span of nub^{1} was very short compared to *OrR,* and the microbial composition shifted dramatically during their life-time. Most remarkably, treating nub^{1} flies with an antibiotic coctail prolonged their life-time survival with more than 100%. Since immune gene expression was elevated in nub^{1} flies also in the presence of antibiotics, it indicates that the early death was not a direct consequence of the aberrant immune gene activity. The shift in microbial composition was characterized by a loss of relatively few operational taxonomic units (OTUs) and a pronounced increase in a large number of *Acetobacter* spp. and *Leuconostoc* spp. We conclude that changes in host genotype, leading to an over-active immune system, can shift the whole microbial community, causing severe dysbiosis and early death.

478C

Study of the Brain Tumor protein role in the midline axon guidance during *Drosophila* neural development. Elise Arbeille, Melissa Hernandez, Jonathan Levin, Greg Bashaw. Department of Neuroscience, University of Pennsylvania, Philadelphia, PA.

During the development of bilateral organisms, commissural axons approach and cross the midline to establish reciprocal connections between the two sides of the CNS. How the commissural growth cone decides to cross and never recross the midline is the subject of intensive research. Indeed, this process is highly conserved and involves various guidance cues and their receptors that instruct axon trajectories towards, within and away from the midline. The DCC family receptor Frazzled (Fra) signals attraction in response to Netrin and also plays a role in down-regulating Slit-mediated repulsion in pre-crossing commissural axons in the *Drosophila* embryonic CNS. Recent studies in our laboratory have shown that neuronal expression of a truncated Fra receptor lacking the entire cytoplasmic domain (FraΔC) results in dose-dependent defects in commissural axon guidance. We used the dose dependent effects of FraΔC expression to screen for additional genes that could work with Fra to promote midline crossing. Brain Tumor (Brat) was identified in this screen. The Brat protein is a translational repressor playing crucial roles in multiple processes during Drosophila early embryogenesis and nervous system development, including the abdominal segmentation, the specification of intermediate progenitor cell identity, the neuromuscular synapse growth and the axon maintenance in the Mushroom Body. Reducing *brat* dosage dominantly enhances the midline crossing defects observed when FraΔC is mis-expressed in the eagle subset of commissural neurons. In addition, in *fra, brat* double mutants, we observe the same severity of midline crossing defects as seen in *fra* single mutants, suggesting that *brat* may act in the same pathway as *fra*. *Brat* gain of function, rescue studies and biochemical experiments are in process to determine how Brat

contributes to Fra-dependent midline attraction. These results promise to define a novel mechanism in the coordination of axon guidance at the midline, involving a new effector, Brain tumor.

479A

Functional requirements of Abelson tyrosine kinase in the nervous system. Han S.J. Cheong, Jessica C. Love, Mark F.A. VanBerkum. Biological Sciences, Wayne State University, Detroit, MI.

Abelson tyrosine kinase (Abl) is a key signaling molecule for axon guidance at the midline of the developing embryonic nervous system. Here, we test the functional requirements of the unique C-terminal region of Abl for rescue of lethality and axon guidance defects in *Abl* mutants. Mutant *Abl* transgenes were created that eliminate kinase activity and/or two regions involved in cytoskeletal remodeling--EVH1-binding motifs (EVH1-BM) and F-actin binding domain (FABD). Ubiquitous expression of kinase-inactive UAS-Abl rescued adult lethality, regardless of the mutations in EVH1-BM and FABD, while ubiquitous expression of kinase-active UAS-Abl induced non-specific lethality. Pan-neuronal expression of these UAS-Abl transgenes in *Abl* mutants indicated that, contrary to our expectations, the EVH1-BM and FABD did not have significant roles in axon guidance, and kinase activity was only partially required. Increasing wild-type Abl levels in *frazzled* mutants induces several guidance defects including a loss of commissures; most of these these defects require kinase activity yet still occur when EVH-BM and FABD are removed. Presently, we are expressing these Abl transgenes in subsets of embryonic neurons to assess subtler cell-autonomous roles for these regions. To date, our data confirms the importance of Abl activity in axon guidance, but suggests that the EVH1-BM and FABD domains are largely unnecessary for this function.

480B

A characterization of growth cone morphodynamics in a live-pathfinding axon. Akanni Clarke[1,2], Ramakrishnan Kannan[1], Irina Kuzina[1], Edward Giniger[1]. 1) NINDS/NIH, Bethesda, MD; 2) The George Washington University.

Live imaging of a single, identified pioneer axon (TSM) extending *in situ* in the developing Drosophila wing reveals a picture of growth cone cell biology that appears divergent from the textbook view of growth cone structure. While we observe image frames that resemble the classic picture of a flat, well-spread, highly ramified growth cone, this is the morph seen when the axon slows dramatically at a choice point as it is searching for guidance information. While TSM is actively extending, its growth cone is narrow with few branches. Indeed, it appears growth cone size is inversely correlated with speed of advance. It is widely thought that lamellipodia provide the mechanical force for growth cone advance. *In situ*, TSM seems nearly devoid of lamellipodia, particularly in the fast-growing morph. It has been proposed that engorgement of a filopodium is the committed step in its transformation to becoming part of the definitive axon. We find that even branches that appear engorged are commonly retracted. Combining our imaging of TSM extension with experiments we have done that order the molecular steps in the Abl signaling pathway suggests a molecular model that would account in detail for the progression of morphologies assumed by the TSM growth cone as it advances in vivo. This gives us a framework to understand growth cone motility and morphogenesis in vivo. We are currently challenging this picture by imaging growth cones lacking single components of the Abl signaling network.

481C

Axon targeting of Gr32a-expressing neurons is dependent on octopamine expression. David Hess-Homeier[1], Gilia Patterson[1], Jessica Bailey[1], Sarah Certel[1,2]. 1) Department of Biological Sciences, University of Montana, Missoula, MT; 2) Neuroscience Graduate Program, University of Montana, Missoula, MT.

Pheromones provide information about gender, receptivity, or conspecificity and are commonly used as olfactory or contact signals in social behaviors like courtship and aggression. Contact pheromones are detected by gustatory receptor-expressing sensory neurons (GRNs), which send axons from the legs or mouth to distinct areas within the suboesophageal zone (SEZ), and other central nervous system sites. In previous work, we demonstrated the existence of functional and putative synaptic connections between Gr32a neurons and octopamine (OA) SEZ neurons. In addition, our results indicate the correct axon targeting by at least a subset of Gr32a neurons is dependent on OA expression. Using *Gr32a-Gal4* to drive reporter GFP expression, we examined the stereotypical projections of Gr32a-expressing neurons from control and OA deficient males. The majority of Gr32a projections reach the SEZ as in controls, however, we observed aberrant termination of Gr32a axons in the antennal lobe region of OA deficient brains that is distinct from previously described projections into the ventrolateral protocerebrum. Here we expand our analysis to examine the consequences of low and high levels of OA on Gr32a synaptic targeting and male aggression. We utilized enhancer trap-flp lines to identify individual Gr32a-expressing neurons that require OA expression and the GRASP technique to determine how synaptic connectivity is altered. Finally, as a subset of OA neurons within the SEZ express the male form of Fruitless (Fru^M), the morphology and pre-synaptic connectivity of OA neurons lacking both Fru^M and OA were examined. As the formation of synapses requires a complex interplay between pre and post-synaptic partners, our experiments address the role biogenic amines play in cell-cell communication and neuronal differentiation.

482A

The transmembrane protein Off-track 2 is implicated in the guidance of embryonic motor neurons. David J Robinson, Samantha Alsbury. University of Greenwich, Medway Campus, Central Avenue, Chatham Maritime, Kent. ME4 4TB. UK.

An essential component of nervous system development entails the projection of axons from neuronal cell bodies to other cells, including other neurons and muscles, resulting in functional circuitry.*Drosophila* have been indispensible in identifying the proteins underlying axon guidance, which depends on receptors on the surface of growth cones detecting attractive and repulsive ligands. A

substantial proportion of this research has utilized embryonic motor neurons, which, in numbering just ~40 per hemisegment, constitute a particularly intelligible system. Bioinformatic analyses of the *Drosophila*proteome have revealed uncharacterized proteins that might be implicated in axon guidance. These possess the same domains as established molecules and are localized to the cell surface. Proteins meeting these criteria that are also paralogous to established axon guidance molecules and that are expressed in similar spatiotemporal patterns are especially promising candidates. Off-track 2, the focus of the current study, is one such candidate, which has recently been shown to co-precipitate with the putative axon guidance molecule, Off-track. The excision of a *P*-element located 28 bp upstream of the 5' UTR of *off-track 2* resulted in the appearance of several nucleotides within the promoter region that differ from those of the parent or wild type sequences. Immunohistochemistry revealed that embryos of this line exhibit highly penetrant phenotypes within a number of motor neuron branches; most notably, the ISNb fails to defasciculate from the ISN in ~75% of hemisegments, though several other branches, including the FB and SB, are absent almost to the same extent. The phenotypes of this line mirror those of embryos with a deficiency spanning the gene, suggesting the altered nucleotides resulted in a loss-of-function, presumably by disrupting transcription. Driving Off-track 2 in somatic musculature caused stalling of the ISNb at various choice points, resulting in reduced innervation of the ventral lateral muscles. While these findings suggest Off-track 2 contributes to the pathfinding behavior of embryonic motor neurons, ongoing work is focused on determining the precise role of Off-track 2 in axon guidance. .

483B

Dissecting the roles of Homeodomain and Hox transcription factors in mediating dendritic development. Sarah G. Clark, Lacey Graybeal, Srividya C. Iyer, Myurajan Rubaharan, Eswar Prasad R. Iyer, Daniel N. Cox. Neuroscience Institute, Georgia State University, Atlanta, GA.

Studies to date, in both vertebrates and invertebrates, have demonstrated that transcriptional regulation functions as an essential control mechanism for mediating dendrite development, with continuing regulatory roles in post-mitotic neurons. *Drosophila* dendritic arborization (da) neurons provide a powerful model for systematic investigations of the molecular mechanisms governing the specification of differential dendritic morphologies across distinct neuronal subtypes. Using a combination of *in vivo* genetics and cellular imaging, we have characterized functional regulatory relationships between the homeodomain transcription factor Cut and the Hox genes, *Antennapedia* (*Antp*) and *Sex combs reduced* (*Scr*), as well as the relationship between these Hox proteins and their putative cofactors in transcriptional regulation, the homeoproteins Homothorax (Hth) and Extradenticle (Exd), in directing cell-type specific dendrite development. Our analyses reveal that Cut regulates, directly or indirectly, the expression of Antp, Scr, Hth and Exd in da neurons and that these molecules function as downstream effectors of Cut-mediated dendritic complexity. Disruption of Antp and Scr results in significant reductions in class III/IV dendritic branching and structure/function studies reveal the transcriptional activation domains of these molecules are essential in regulating class-specific dendrite homeostasis. Functionally, Hth and Exd are known to act as hetero-dimers in directing Hox-mediated gene expression in other cell types. Interestingly, our analyses reveal differential distributions of these two homeoproteins in distinct da neuron subclasses suggesting that differential regulation of Hth and Exd expression may be essential for specification of class-specific dendritic architectures.

484C

HSPG-dependent Regulation of Dendrite Development. Amy R Poe, Chun Han. Graduate Field of Genetics, Genomics, and Development, Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Dendrites are cell body extensions of neurons responsible for collecting synaptic and sensory information. The normal function of a nervous system requires its neurons to develop proper dendritic fields and branching patterns. Disorders such as Down's syndrome, schizophrenia, Angelman's syndrome, and autism have been linked to defects in dendrite development and maintenance. How do neurons develop dendritic arbors with the proper branching patterns in a complex nervous system? What developmental signaling pathways contribute to dendrite development? *Drosophila* dendritic arborization (da) neurons provide a powerful model system to understand the developmental signaling pathways involved in dendrite development. The dendrites of da neurons develop in a complex cellular environment and are influenced by surrounding cells. However, the mechanisms by which surrounding cells influence dendrite growth are poorly understood. Heparan sulfate proteoglycans (HSPGs) are cell-surface and extracellular glycoproteins that carry heparan sulfate sugar chains. HSPGs play key roles in cell signaling and extracellular transport of growth factors such as Wnts, Hh, and EGFs. To investigate the potential roles of HSPGs in dendrite development, we knocked down HS chain biosynthesis genes *sfl*, *botv*, and *ttv* in epidermal cells. Loss of HS led to a drastic reduction of class IV da neuron dendrites, indicating that HSPGs are required for dendrite development. *Drosophila* have four HSPG proteins including Syndecan (Sdc), Glypicans (Dally & Dally-like), and Perlecan (Trol). To determine which HSPG genes are important for dendrite development, we examined dendrite phenotypes in HSPG homozygous mutant larvae and in animals where HSPG genes were knocked down. These experiments suggest that HSPG genes play redundant roles in regulating dendrite growth. In addition, overexpression experiments show that high levels of HSPGs inhibit dendrite growth. Our data demonstrate the complex roles of HSPGs in regulating dendrite development of class IV da neurons. Additional experiments will further show how HSPGs may regulate dendrite growth through their core proteins and HS chains.

485A

FOXO Regulates Dendrite Branching. J. Sears, H. Broihier. Neurosciences, Case Western Reserve University, Cleveland, OH.

Neuronal morphology is a key determinant of neuronal function. The stereotyped morphologies defining distinct neuronal populations result from the actions of cytoplasmic and signaling proteins that together define cell shape. The transcription factors that coordinate the expression of these effectors are critical to identify, yet poorly defined. FOXO is an evolutionarily-conserved transcription

factor previously shown by our lab to promote a dynamic microtubule (MT) network at the neuromuscular junction.
To test whether FOXO also regulates dendritic morphogenesis, we set out to analyze dendritic morphology in FOXO mutant backgrounds. We focused on the dendritic arborization (da) neurons. There are four classes of these sensory neurons, each with characteristic positions and morphologies. We discovered that FOXO is necessary for dendrite branching. Moreover, differential effects on MT-rich and actin-rich branches implicate FOXO in cytoskeletal regulation. FOXO nulls display marked reductions in numbers of dendrite terminals, branch points, and total length in all classes of da neurons. In class IV, this reduction is most apparent in higher order (closer to endpoint) branching, resulting in cells that are less densely packed in their coverage, but with comparatively normal main branches. Interestingly, FOXO nulls have longer terminal branches in class III cells. These branches are actin-rich and MT-poor. These loss-of-function phenotypes differ strikingly from that observed in FOXO-overexpressing neurons. FOXO overexpression drives an increase in short, MT staining negative branches in class I, but also a drastic loss of higher order branching in class IV, leaving mostly short terminal branches attached to main branches. In line with these observations, growing class IV cells in FOXO null animals have longer terminal branches. Based on these data, we conclude that FOXO plays a role in dendrite development, and that this is likely due to changes in cytoskeletal regulation. We are working to determine when and where FOXO is acting, and how the cytoskeleton is altered in response to changes in FOXO levels. We are also screening for additional members of a FOXO-dependent molecular pathway to further explore this key pathway.

486B

Short stop exerts differential effects on class-specific dendritic homeostasis. Sarah Trunnell, Shatabdi Bhattacharjee, Daniel N. Cox. Neuroscience Institute, Georgia State University, Atlanta, GA.

Spectraplakins are an evolutionarily conserved family of cytoskeletal cross-linking proteins that provide a physical link between the F-actin and microtubule cytoskeletons. As cytoskeletal structure and organization are key mediators of neural shape, and by extension neural function, understanding the molecular mechanisms underlying the regulation of cytoskeletal dynamics is essential to achieving insight into the homeostatic process of subtype-specific arborization. While the roles of the sole *Drosophila* Spectraplakin, *short stop* (*shot*), have been well described in axonal development, putative functional roles of Shot in dendritogenesis remain incompletely understood. We address this knowledge gap by conducting systematic genetic and structure-function mutant analyses to explore the functional role(s) of Shot in dendritic development using da sensory neurons as a model system. Expression studies reveal Shot localization to all da neuron subclasses (I-IV), including enrichment at dendritic branch points and termini. Despite this uniform expression, mutant phenotypic analyses indicate that Shot exerts context-dependent effects on subtype-specific dendritic arborization, whereby Shot restricts dendritic complexity in morphologically simple neurons, while promoting branching and growth among morphologically complex neurons. Given the cytoskeletal cross-linking role of Spectraplakins, as well as the differential distribution of F-actin and microtubule-based cytoskeletal fibers in distinct neuronal subtypes, we investigated how disruptions in Shot functional domains would not only affect Shot subcellular localization, but also impact dendritic architecture and the expression/organization of the neuronal cytoskeleton. These studies identified specific domains required for Shot subcellular trafficking and/or localization together with roles in modulating distinct aspects of arbor development. Finally, we have elucidated a putative role of Shot in anchoring of polarized secretory trafficking machinery (ER/Golgi) in dendrites. Collectively, these studies provide new insights into the roles of Spectraplakins in dendrite morphogenesis, cytoskeletal organization, and regulation of the secretory machinery.

487C

Endocytic Pathways Downregulate the L1-type Cell Adhesion Molecule Neuroglian to Promote Dendrite Pruning in *Drosophila*. Heng Zhang[1], Yan Wang[1,2], Jack Jing Lin Wong[1,2], Kah-Leong Lim[3,4,5], Yih-Cherng Liou[1], Hongyan Wang[2,3,5], Fengwei Yu[1,2,3]. 1) 1Temasek Life Sciences Laboratory and Department of Biological Sciences, National University of Singapore, 1 Research Link, Singapore 117604, Singapore; 2) NUS Graduate School for Integrative Sciences and Engineering, Centre for Life Sciences, Singapore 117456, Singapore; 3) Neuroscience and Behavioural Disorders Program, Duke-NUS Graduate Medical School, Singapore 169857, Singapore; 4) National Neuroscience Institute, Singapore 308433, Singapore; 5) Department of Physiology, National University of Singapore, Singapore 117597, Singapore. Pruning of unnecessary axons and/or dendrites is crucial for maturation of the nervous system. However, little is known about cell adhesion molecules (CAMs) that control neuronal pruning. In *Drosophila*, dendritic arborization neurons, ddaCs, selectively prune their larval dendrites. Here, we report that Rab5/ESCRT-mediated endocytic pathways are critical for dendrite pruning. Loss of Rab5 or ESCRT function leads to robust accumulation of the L1- type CAM Neuroglian (Nrg) on enlarged endosomes in ddaC neurons. Nrg is localized on endosomes in wild-type ddaC neurons and downregulated prior to dendrite pruning. Overexpression of Nrg alone is sufficient to inhibit dendrite pruning, whereas removal of Nrg causes precocious dendrite pruning. Epistasis experiments indicate that Rab5 and ESCRT restrain the inhibitory role of Nrg during dendrite pruning. Thus, this study demonstrates the cell-surface molecule that controls dendrite pruning and defines an important mechanism whereby sensory neurons, via endolysosomal pathway, downregulate the cell surface molecule to trigger dendrite pruning.

488A

Examining the Effects of Oxidative Stress on the Development of a Defined Neural Circuit in *Drosophila melanogaster*. Ryan Doan, Parag Bhatt, Wendi Neckameyer. Pharmacological & Physiological Science, Saint Louis University School of Medicine, Saint Louis, MO.

Disruptions in neuronal function resulting from improper central nervous system development and maintenance have been implicated in a wide array of neurological diseases, such as Parkinson's disease. Oxidative stress has been shown to damage neural fibers in

Parkinsonian models via an increase in reactive oxygen species; paraquat is the canonical method by which to induce oxidative stress in many models including the fruit fly, *Drosophila melanogaster*. We have employed the stomatogastric feeding circuit in *Drosophila* larvae to correlate the functional output, feeding, with development changes in the axonal architecture innervating the foregut (Neckameyer, 2010). We exposed *Drosophila* larvae to paraquat for 24 hours to induce acute oxidative stress, and examined the effects on both feeding and neurite architecture of the foregut. At doses 30mM and below, we observed a depression in feeding without other obvious physiological impairments, as well as a significant increase in the number and size of serotonergic presynaptic vesicles along the neurite length. We will use this model to assess the involvement of oxidative stress on neural circuitry development.

489B

Autophagy affects glutamate receptor localization at the *Drosophila* neuromuscular junction. Elizabeth Isbell, Faith Libel. Biological Sciences, Southern Illinois University-Edwardsville, Edwardsville, IL.

Glutamate, one of the most abundant neurotransmitters, is responsible for fast excitatory neurotransmission. Glutamate receptors (GluRs) have an important role in learning and memory and synaptic plasticity can occur through the insertion or removal of postsynaptic glutamate receptors. We have found that autophagy-specific genes (*atg)* influence the localization of glutamate receptors at the*Drosophila* neuromuscular junction. Mutations of *atg1* and *atg8a* result in a significant decrease in postsynaptic GluRs, while cellular levels of GluR protein remain unaffected. Rescue experiments indicate*atg1* is required in neurons and muscle cells, while restoration of *atg1* in glial cells did not rescue GluR localization. To determine the mechanism by which the Atg proteins regulate GluR localization, we are assessing if pharmacological inhibition of autophagy affects GluR localization. In addition, we are trying to determine if inhibition of autophagy affects glutamate metabolism thereby altering postsynaptic GluR localization. The results from these experiments will give further insight as to autophagy's role in glutamate receptor trafficking and localization. .

490C

TDRD3 works with an RNA topoisomerase and Fragile X mental retardation protein to promote synapse formation. S. Lee[1], W. Shen[1], Y. Xue[1], D. Xu[1,3], S. Zou[2], W. Wang[3]. 1) Lab of Genetics, National Institute on Aging, National Institute of Health, Baltimore, MD 21224; 2) Translational Gerontology Branch, National Institute on Aging, National Institute of Health, Baltimore, MD 21224; 3) College of Life Sciences, Peking University, Beijing, China.

Topoisomerases are essential to resolve the topological problems in DNA metabolism, but it remains unclear whether they are also needed for RNA metabolism. Recently, we have identified human topoisomerase 3b (Top3b) as the first topoisomerase for RNA in eukaryotes. We and others have demonstrated that Top3b is critical for neural development and mental health, as *top3b* mutation in humans is linked to schizophrenia and intellectual disability, whereas inactivation of Top3b in mouse and *Drosophila* results in abnormal synapse formation. Top3b forms a complex with TDRD3 (tudor-domain containing 3), and this complex biochemically and genetically interacts with Fragile X syndrome mental retardation protein, FMRP. While Top3b and FMRP are known to be essential for normal neurodevelopment, the role of TDRD3 in this process remains unclear. Here we show that the Top3b forms a similar complex with TDRD3 in*Drosophila*, and this complex also interacts with FMRP. In addition, *tdrd3* mutant flies have abnormal synapse synaptic formation similar to that observed in *top3b* and *fmr1* mutant flies. Moreover, TDRD3 genetically interacts with Top3b in an antagonistic manner during synapse formation. In human cells, TDRD3 is required for Top3b to bind FMRP, to associate with polyribosomes, and to localize in RNA stress granules. Together, these data suggest that the Top3b-TDRD3 complex works coordinately with FMRP to promote synapse formation and mental health.

491A

Kismet affects synaptic transmission and endocytosis at the *Drosophila* Neuromuscular Junction. Carley Gridley, Taylor Delaney, **Faith Liebl**. Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL.

Efficient synaptic communication requires bidirectional signaling between the pre- and postsynaptic cells. This signaling depends on the localization of synaptic proteins to mediate endo- and exocytosis at appropriate synaptic locations. We have found that the chromodomain helicase DNA binding (CHD) protein, Kismet (Kis), which regulates chromatin remodeling, influences synaptic transmission and, possibly secondarily, endocytosis. Animals with mutations in *kis* exhibit a significant decrease in the frequency and amplitude of spontaneous neurotransmission and in evoked amplitudes. The change in neurotransmission may be at least partly due to a reduction in endocytosis in *kis* mutants. Further, *kis* mutants exhibit a change in the localization of the postsynaptic glutamate receptor subunit, GluRIIC, relative to Brp, a protein localized to active zones. Our data suggests that Kis affects synaptic transmission and endocytosis by regulating distinct processes in both the pre- and postsynaptic cells. Based on these data, we hypothesize that Kis regulates synaptic transmission, possibly by influencing transcription of synaptic target genes involved in bidirectional synaptic signaling and/or endocytosis. We are currently assessing whether Kis regulates synaptic transmission by recruiting Ash1 or by inhibiting the function of the polycomb group complex.

492B

MicroRNA-8 promotes robust motor axon targeting by coordinate regulation of cell adhesion molecules during synapse development. Cecilia Lu[1,2], Bo Zhai[2], Alex Mauss[3,4], Matthias Landgraf[3], Steve Gygi[2], David Van Vactor[1,2]. 1) Okinawa Institute of Science and Technology, Onna-son, Okinawa, Japan; 2) Department of Cell Biology, Harvard Medical School, Boston, Massachusetts, USA; 3) Department of Zoology, University of Cambridge, Cambridge, UK; 4) Max Planck Institute of Neurobiology, Martinsried, Germany.

Synapse morphogenesis is a finely orchestrated process that requires spatiotemporal coordination between both pre- and post-

synaptic cells to initiate and maintain precise contacts throughout development. Cell surface proteins and adhesion molecules (CAMs) play critical roles in synapse formation and stabilization but the mechanism by which such molecules are regulated at post-transcriptional level is not clear. We uncovered a conserved microRNA miR-8 from a forward genetic screen in *Drosophila* as a functional component in the regulation of synaptic growth and morphology. We found that miR-8 null mutants display defects in the earliest stage of target refinement at the neuromuscular junction (NMJ). Using the microRNA silencing P element (miR-SP) method, we showed that miR-8 regulates motor axon path-finding and synaptic morphology by acting on both pre- and post-synaptic targets. Interestingly, multiple CAMs including Neuroglian (Nrg), the *Drosophila* homolg of the vertebrate L1 cell adhesion molecule (L1CAM) and Fasciclin 3 are down-regulated by more than 2 folds in the miR-8 mutant as quantified by using stable isotope labeling of cells (SILAC) and mass spectrometry. While pre-synaptic or post-synaptic overexpression of Nrg partially rescued the miR-8 mutant phenotype, Nrg and Fas3 genetically interacted to recapitulate miR-8 effects on synapse morphogenesis. Since microRNA are short (~22 nt) non-protein coding RNA with pervasive functions in biology through post-transcriptional regulation of gene expression, our study presents miR-8 as a regulator in synapse morphogenesis and postulates a potential mechanism to investigate the coordinated assembly of pre- and post-synaptic compartments during the development of nervous system. .

493C

Neto-mediated intracellular interactions shape postsynaptic structures at the *Drosophila* neuromuscular junction. Cathy Ramos, Oghomwen Igiesuorobo, Qi Wang, Mihaela Serpe. NICHD, NIH, Bethesda, MD.

At synaptic sites, ionotropic glutamate receptors (iGluRs) require postsynaptic structures enabling their clustering and stabilization, thus ensuring efficient neurotransmission. At the *Drosophila* glutamatergic neuromuscular junction (NMJ), iGluRs are composed of three shared subunits (GluRIIC,D or E) and either -A or –B, meaning type-A or type-B iGluRs. Our studies examine how the iGluRs clustering is developmentally coordinated with the establishment of postsynaptic components. At NMJ, the iGluRs clustering absolutely requires an auxiliary subunit, Neto (Neuropilin and Tolloid-like protein).*Drosophila neto* locus encodes for two isoforms, Neto-α and Neto-β, that are generated by alternative splicing, only differing by their intracellular parts. We found that the Neto-mediated iGluRs clustering resides in the shared domains of the isoforms, while the specific *neto-β* mutation alters the recruitment of type-A iGluRs. We generated *neto*-β isoform specific alleles, a *neto*-β genetic null and a truncated*neto*-β, lacking a part of the cytoplasmic tail. Both alleles show partial lethality and exhibit small NMJs with physiological defects. They have decreased amplitudes of mini-extrajunctional potentials, which correlate with a reduction of synaptic type-A iGluRs levels. However, they have normal evoked junctional potentials due to a presynaptic compensation sustained by an increase of synaptic contacts. Electron micrographs show reduced postsynaptic densities (PSDs) and subsynaptic reticulum. Moreover, *neto*-β alleles have decreased signals for postsynaptic proteins such as p21-activated kinase (PAK), which is crucial for synaptic stabilization of type-A iGluRs. We show that Neto-β controls the recruitment and the stabilization of PAK at PSDs. Importantly, either Neto isoform (α or β) can rescue the embryonic lethality of $neto^{null}$ animals when expressed in the striated muscle, indicating that the Neto-mediated iGluRs clustering resides in the shared domains. However, only Neto-β isoform can recruit PAK at the NMJs. Thus, by coordinating type-A iGluRs and PAK synaptic recruitment, Neto-β couples the iGluRs arrival at synaptic sites with postsynaptic differentiation enabling structural and functional synaptic plasticity.

494A

The Ig Transmembrane Protein Borderless Is Required for Synaptic Development and Function in the Drosophila Visual System. Hunter S. Shaw, Scott Cameron, Wen-Tzu Chang, Yong Rao. Centre for Research in Neuroscience, Department of Biology, Department of Neurology and Neurosurgery, McGill University, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada.

Development of the *Drosophila* melanogaster visual system is an excellent model to understand molecular and cellular mechanisms controlling neural network formation during embryonic development. The*Drosophila* visual system is comprised of the compound eye and optic lobe. The compound eye consists of ~800 ommatidia or single eye units, each containing eight photoreceptor cells (R cells). R1-R6 axons synapse in the superficial lamina, while the R7 and R8 axons target the deeper medulla layer. Once R-cell axons reach their target in the brain, they must recruit the proper presynaptic machinery in order to establish fully functional synapses. Recent studies in our lab identified a novel Ig transmembrane protein Borderless (Bdl). Bdl mediates homophilic cell-cell adhesion in vitro, and down-regulation of Bdl is required for R7 axonal tiling and layer-specific target selection. In this study, we show that Bdl is also required for the assembly of pre-synaptic machinery in R8 axons. Genetic mosaic analysis and cell-type-specific rescue indicate that Bdl is required in both R8 axon and its target region. We are performing experiments to elucidate the exact mechanism by which Bdl regulates synaptic development and function. The results will be presented at the meeting.

495B

Conserved interactions between Sorting nexins and Nervous wreck reveal a role for SH3PX1 at synapses. Fiona P. Ukken, Joseph J. Bruckner, Kate O'Connor-Giles. UW-Madison, Madison, WI.

Growth factor signaling pathways are key regulators of synaptic growth. Multiple endocytic proteins have been shown to regulate synaptic growth at the Drosophila neuromuscular junction (NMJ) through the modulation of these pathways. Nervous wreck (*nwk*), a neuron-specific F-Bar protein, interacts with Dynamin, Dap160 and Wasp to attenuate BMP signalling and regulate synaptic growth and function. To understand the conserved molecular mechanism through which Nwk functions, we investigated the highly conserved mammalian ortholgues of *Drosophila* Nwk, Nwk1 and Nwk2. Both proteins are expressed in the cerebral cortex and hippocampus at embryonic and early post-natal stages. Nwk 1 expression diminishes by postnatal day 16, while Nwk2 expression is maintained during later stages when most synaptic growth occurs. To identify conserved interactors that may function with Nwk in the regulation of

synaptic development, we used an immunoprecipitation/mass spectrometry-based approach and identified Snx9 and18 as Nwk2 interactors. Further experiments showed that the third member of the Snx9 family, Snx33, also binds Nwk2. We find that this interaction is conserved in *Drosophila*, where a single homologue, SH3PX1, represents the Snx9 family. Using CRISPR-Cas9 genome engineering, we generated endogenously tagged SH3PX1 fly lines. Consistent with a functional interaction at synapses, SH3PX1 is expressed both pre- and post-synaptically at the larval NMJ and colocalises with Nwk in the presynaptic periactive zone. Using a similar genome engineering approach, we generated null alleles of SH3PX1 and find that SH3PX1 functions with Nwk in the regulation of synaptic growth and function. These experiments demonstrate the feasibility of combining biochemical studies in mammalian cells with CRISPR-Cas9-mediated functional studies in Drosophila to identify conserved mechanisms of nervous system development and function.

496C

Separable intrinsic and extrinsic timers regulate terminal differentiation of a target-dependent gene in *Drosophila* neurons. Anthony Berndt[1], Jonathan Tang[2], Tianshun Lian[1], Ridyard Marc[1],**Douglas Allan**[1]. 1) Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Genetics, Harvard Medical Schol, 77 Avenue Louis Pasteur, Boston, MA 02115.

Regulation of neuronal terminal differentiation often requires a target-derived signal, but is this signal a simple temporal cue, and is it permissive or instructive for differentiation? *Drosophila* Tv4 neurons require a target-derived BMP signal to initiate *FMRFa* expression. Here, dissection of *FMRFa cis*-regulation reveals essential roles for two non-overlapping ~30bp *cis*-elements; a Smad-binding BMP-response element (BMP-RE) and an Apterous-binding homeodomain-response element (HD-RE). We hypothesized these would synergize spatial information (HD-RE), with an on/off BMP temporal switch (BMP-RE). However, both short *cis*-elements contain sufficient information for restricted Tv4 expression and also correct temporal activation. In addition, we identified a second temporal cue which appears to act as an intrinsic timer to prevent early activation of both HD-RE and BMP-RE enhancers. These data indicate that neuronal terminal differentiation can involve separable double assurance mechanisms for both spatial and temporal gene expression.

497A

***Antp* controls the differential survival and morphology of adult-specific neurons in the thoracic vs. subesophageal ganglia. Kathleen Bell**, Christine Murphy, Amy Patterson, Ginna Freehling, Alexandra Panzarino, Erin Schuler, Elizabeth Marin. Biology Department, Bucknell University, Lewisburg, PA.

Developmental patterning genes sculpt adaptive variations on a basic nervous system plan by altering neuronal survival patterns and morphology. Within each segment of the *Drosophila* postembryonic ventral nervous system, twenty-five neuroblasts generate distinct lineages of adult-specific neurons. Each lineage consists of two different hemilineages that result from the accumulation of two distinct sibling cell types. Many of these hemilineages display segment-specific survival and morphology in the thorax and upper abdomen. Previously, we have shown that the Hox gene *Ubx* is expressed in a segment-, lineage-, and hemilineage-specific manner in the posterior third thoracic and first abdominal segments. Moreover, *Ubx* is both necessary and sufficient to confer segment-appropriate survival and morphology in these neurons. In many cases, expression of *Ubx* results in neuronal death, but in others, it is required for neuronal survival. These findings suggest that *Ubx* has been evolutionarily co-opted for distinct roles in different neurons. However, it is not yet clear whether *Ubx* acts directly in these patterning functions, or whether it interacts with its anterior neighboring Hox gene, *Antp*. *Antp* is expressed most strongly in the second thoracic segment and the anterior portion of the third thoracic segment, again in a lineage- and hemilineage-specific manner. Loss of *Antp* results in the anterior transformation of neurons in the thorax, while gain of *Antp* in the subesophageal ganglion results in posterior transformations. Notably, these alterations affect segment-appropriate axon projection patterns as well as cell survival. Currently, we are investigating the interactions between *Ubx* and *Antp* during development that may determine segment-appropriate survival and morphology.

498B

Genetic regulation of cell fate determination in the *Drosophila* ocelli. A.K. Mishra, S.G. Sprecher. Department of Biology, University of Fribourg, Fribourg, Switzerland.

How equipotent precursor cells acquire distinct fates remains a major question in developmental biology. The visual system of *Drosophila* is an impacting model to study cell fate determination. Photoreceptors (PRs) are sensitive to a certain wavelength of light depending on which *rhodopsin (rh)* gene they express. While cell fate determination in the compound eye has been extensively studied, virtually nothing is known regarding the development of the ocelli (or simple eyes). Each ocellus contains about 80-100 PRs; all of them belong to one cell type and express UV-blue sensitive Rh2. While specification of ocellar PRs is mediated by the combinatorial action of *homothorax (hth)*, *twin of eyeless (toy)*, retinal determining genes and signaling molecules, it is still unknown as to how these PRs are orchestrated during development to acquire ocellar PR cell fate. We found that *hth* is expressed in the ocellar PRs and is both necessary and sufficient for the differentiation into the ocelli-specific cell type, which expresses Rh2. Hth, together with Extradenticle (Exd), regulates cell identity of ocellar PRs by promoting expression of Rh2 and repressing Rh1, a rhodopsin normally expressed in the outer PRs of the retina. Intriguingly, misexpression of Hth in the compound eye results in the ectopic expression of Rh2. Our findings suggest that Hth acts as a genetic switch between an Ocellar-PR-type versus Outer-PR-type. We also provide an evidence of how transcriptional networks are responsible for the determination of a particular cell type during development.

499C

A temporal series of transcription factors in mushroom body precursors. Anthony M Rossi, Claude Desplan. Biology, New York University, New York, NY.

How multitudes of different neuronal types are produced during development is still poorly understood. The *Drosophila* mushroom body (MB) is a unique model to study this problem because MB function and development are well described. The four cell types that comprise it are sequentially produced from four identical neuroblasts (NBs) that divide continuously during development. However, the molecular mechanisms controlling the temporal production of MB neurons remain enigmatic. In *Drosophila* ventral nerve cord and optic lobe NB lineages, transcription factors (TFs) are sequentially expressed and then inherited by post-mitotic progeny to determine different cell fates. We hypothesize that a TF cascade acts in MB NBs to define the successive cell fates they produce. We are screening for TF expression in the MB throughout *Drosophila* development to identify the TFs defining the four MB neuron types (i.e., γ, α'β', pioneer αβ and αβ neurons). We have currently identified three TFs expressed in MB precursors when γ and αβ neurons are born, neurons that function in short and long-term memory, respectively. Interestingly, *Drosophila* MB neurogenesis shares many properties with vertebrate neural development. For example, stem cells in the vertebrate retina and cortex also produce neurons sequentially. Our MB studies could shed light on general modes of neurogenesis and have broader impacts on brain function, such as learning and memory. .

500A

Investigating the Transcriptional Programs that specify Axon Targeting of Adult Leg MNs. Lalanti Venkatasubramanian, Jonathan Enriquez, Richard Mann. Columbia University Medical Center, New York, NY.

The ability of animals to perform coordinated movements depends on the precise organization of neural circuits controlling motor function. One of the key components of these circuits are the motor neurons (MNs), which execute coordinated movements by forming distinct connections between the CNS and periphery. Each MN is genetically encoded to develop and maintain a unique identity, which is defined, in part, by their morphology. However the transcriptional programs and effector genes controlling MN morphologies are still unknown. Using *Drosophila melanogaster*, our lab has shown that the Hox gene *Antennapedia* (*Antp*) specifies the axonal targeting of adult leg MNs in a level dependent manner (Baek *et al.,*2013). In this study we describe the function of another transcription factor, Zinc finger homeodomain 1 (Zfh1) which is expressed in a subset of adult leg MNs. Our results show that Zfh1 also plays a role in specifying axon trajectories of leg MNs. We further test the hypothesis that Antp and Zfh1 genetically interact with each other to control axon targeting of MNs to distinct regions in the leg.

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501B

RNA stability regulates balance between neuroblasts division and differentiation. Lu Yang[1], Richard Parton[1], Tomasz Dobrzycki[1], Yoav Arava[2], David Ish-Horowicz[3], Ilan Davis[1]. 1) Biochemistry Dept., University of Oxford, Oxford, United Kingdom; 2) Department of Biology, Technion, Israel Institute of Technology, Haifa, Israel; 3) MRC for Molecular Cell Biology, University College London, London, United Kingdom.

Brain development relies on the precise regulation of the balance between neural stem cell proliferation and differentiation. Neuroblasts asymmetrically divide and give rise to two daughter cells. The fate of daughter cells depends on the expression level of specific protein regulators. One most extensively studied regulator is Prospero (pros). If the Pros protein level is kept low then this cell remains a proliferating neuroblast. If Pros protein is upregulated, this induces the daughter cell to terminally divide and differentiate. Therefore, the expression level of Prospero is crucial for brain development. However how the expression level of Prospero and other protein regulators is regulated in different cell types remains largely unknown. In this study, we attempt to dissect this regulatory molecular mechanism, focusing on two well-studied regulators *pros* and *brat*. With a combination of biochemical and immunofluorescent techniques, we showed *pros* and *brat* RNA are transcribed in both the neuroblasts and the daughter cells but the stability of the RNA is differentially regulated in the different cell types, therefore protein expression of *pros* and *brat* are regulated at a post-transcriptional, RNA stability level. We believe this is of biological significance because we have identified an upstream regulator of *pros* and *brat* mRNA stability called Syncrip (syp). In *syp* mutants, we found significantly enlarged optic lobes due to neuroblast over proliferation, similar to *pros* and *brat* mutants. Interestingly, we showed *pros* and *brat* mRNAs are unstable in *syp* mutants but the transcription of *pros* and *brat* and not affected. Our results suggest Syncrip works to maintain *pros* and *brat* expression in the daughter cells by preventing RNA degradation by maintaining the poly-A length of its RNA targets. This work could be the foundation of a novel mechanism for achieving the balance of neural stem cell division and proliferation through the regulation of RNA stability.

502C

How does the misexpression of CHMP2B affect the wing development of *Drosophila melanogaster*? Pralaksha Gurung, Andrew Rhoads, S. Tariq Ahmad. Colby College, Waterville, ME.

The CHMP2B is a coding sequence that functions in the recycling and degradation of cell surface receptors. It is expressed in neurons in all major regions of the brain. In an earlier study, this sequence has been misexpressed in the eye of the *Drosophila melanogaster*. It has been established that this code plays a fundamental role in the development of the eyes for the *Drosophila*. The aim of this study is to examine if the CHMP2B code effects can be expressed in the wings of the *Drosophila*. This is done so by driving the UAS-CHMP2B with different Gal4 lines. Our preliminary results have shown that the coding sequence appears to cause the disjunction of the wing margin, as well as contributes to the deformation of the veins on the wings. The effect of the CHMP2B appears to differ from one Gal4

line to other. We are currently expanding our Gal4 lines to further inspect the effects. Results are to be discussed.

503A

***Drosophila* miR-34 overexpression results in neural remodeling defect of mushroom body γ neurons. Yen-Wei Lai**[1,2], Hung-Hsiang Yu[3], Chun-Hong Chen[2]. 1) Molecular and Cellular Biology, Taipei, Taiwan; 2) Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Taiwan; 3) 3.Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan.

The learning and memory in *Drosophila* are regulated by mushroom bodies (MB), which composed of three different types of neurons: γ, α'β' and αβ. MB neurons also called kenyon cells because their axons bifurcate two lobes: dorsal lobe and medial lobe. MB neuroblasts produce γ neurons from embryogenesis to mid-third-instar larval stage, and then γ axons and dendrites are remodeling after puparium formation. However, the mechanism of neural remodeling is still unclear. GAL4/UAS binary system in Drosophila is well-established tool allowing us to overexpress genes or microRNAs in order to observe biological functions in specific tissues and/or cells. A previous study showed that *Drosophila* miR-34 is mainly expressed in the brain during adult stage and plays an important role in neural degeneration and life span by down-regulating *E74*. In order to address the function of miR-34 in neural development, we overexpressed miR-34 in MBs, which results in γ neuron remodeling defect. We wonder whether γ neuron remodeling shares the similar mechanism as neural degeneration through the miR-34/E74-mediated pathway. However, through similar overexpression experiments, we ruled out the involvement of miR-34 targets, E74, Hr4 and Yema, in the regulation of γ neuron remodeling. Our study suggested that miR-34 regulates γ neuron remodeling through a different mechanism as compared to miR-34 in neural degeneration. Further study will be done to figure out possible molecules that regulates γ neuron remodeling.

504B

Kinesin-1-powered microtubule sliding drives axonal regeneration in *Drosophila* cultured neurons. Wen Lu, Margot Lakonishok, Vladimir Gelfand. Department of Cell and Molecular Biology, Northwestern University, Feinberg School of Medicine, Chicago, IL.

Understanding the mechanism underlying axon regeneration is of great practical importance to develop therapeutic treatment of traumatic injuries of brain and spinal cord. Dramatic cytoskeleton reorganization occurs at the injury site, and microtubules have been implicated in the regeneration process. Previously we demonstrated that microtubule sliding by conventional kinesin (Kinesin-1) is required for initiation of neurite outgrowth in *Drosophila* embryonic neurons, and that sliding is developmentally downregulated when neurite outgrowth is completed. Here we report that mechanical axotomy of*Drosophila* neurons in culture triggers axonal regeneration and regrowth. Regenerating neurons contain actively sliding microtubules; this sliding, like sliding during initial neurite outgrowth, is driven by Kinesin-1 and is required for axonal regeneration. The injury induces a fast spike of calcium, and local depolymerization of microtubules and subsequent formation of microtubule arrays with mixed polarity. These events are required for reactivation of microtubule sliding and regeneration. Furthermore, c-Jun N-terminal kinase (JNK) pathway promotes regeneration by enhancing microtubule sliding in injured mature neurons.

505C

Gap junctions are required for glia-glia communication and survival in *Drosophila* PNS. M. Das, V. Auld, C. Rankin. University of British Columbia, Vancouver.

Glia perform many crucial functions that are necessary for the development of the nervous system. In *Drosophila* PNS, axons are ensheathed by three types of glia. Innermost glia called wrapping glia (WG) directly ensheath individual axons. This layer is surrounded by the subperineurial glia (SPG) which encircles the entire nerve bundle and also forms the blood-nerve barrier (BNB). Lastly, the perineurial glia (PG) wraps around subperineurial glia and an extracellular matrix surrounds these glial layers. At present, how these glia communicate with each other and what molecular cues and signaling is required for proper ensheathment of axons is unknown. Direct cell-cell communication can occur via channels formed by gap junctions (GJ) proteins. We show that an invertebrate gap junction protein, Innexin 2 (Inx2) is present in all three glial cells of larval *Drosophila* PNS. Downregulation of Inx2 specifically in all glial cells by expressing RNA interference (RNAi) transgenes leads to lethality at larval stages, implying that gap junctions in glia are important for the survival of the fly. Inx2 null mutants are embryonic lethal and expression of tagged and untagged Inx2 transgenes in glia was not sufficient to rescue embryonic lethality. This may be due to Inx2 function in other tissues. We show that targeted downregulation of Inx2 in the subperineurial glia results in a collapsed wrapping glia. This suggests that gap junctions are required for the subperineurial glia and wrapping glia to communicate. Although, downregulation of Inx2 in the wrapping glia also leads to collapsed wrapping glia, the wrapping glia is unaffected by the downregulation of Inx2 in the perineurial glia. Moreover, Inx2 downregulation in the subperineurial glia results in TUNEL positive glia suggesting that these cells are dying. To determine if this affects the physiology of the larva, they were tracked for locomotor deficits. Using the GAL80^{ts} system we also show that Inx2 is important for early development but not maintenance of the glial sheath. In conclusion, we have demonstrated that Inx2 is important for glia-glia communication, the loss of which leads to glial cell death and lack of proper glial ensheathment during development. .

506A

An evolutionarily conserved gene function underlying the Glial Regenerative Response in the living injured larval CNS. Maria Losada-Perez, Neale Harrison, Alicia Hidalgo. Biosciences, University of Birmingham, Biermingham, Birmingham, United Kingdom.

Regeneration after injury occurs in many animals, revealing that cellular and molecular mechanisms regulate normal organ structure, size and shape. The human central nervous system (CNS) does not regenerate upon damage and as a consequence stroke, spinal cord injury, multiple sclerosis and neurodegenerative diseases result in permanent disability. However, glial cells respond to CNS injury by

proliferating to produce more ensheathing glia and trophic factors that favor axonal growth. This glial regenerative response is found across the animals, from cockroaches and flies, to fish and mice, implying that there is an underlying genetic mechanism that has been evolutionarily conserved. A balanced control of proliferation and differentiation is necessary to ensure repair and prevent unwanted outcomes (e.g. glioma), however how this comes about is little understood. *Drosophila* is a powerful model organism to discover gene networks and test gene function *in vivo*, and has recurrently led to discoveries relevant to human health. Our lab discovered a gene network in *Drosophila* that can be manipulated in glia to promote CNS repair. Essentially, two feedback loops involving Prospero, Notch and NFkB control the differentiation and proliferation of glial cells. We showed that manipulating this gene network could shift the response to injury of glial cells from promotion to prevention of repair. However, this took place in the dissected CNS. We have established a new injury paradigm in the living larva, which will enable us to also investigate functional recovery of behaviour. We will present new data on an evolutionarily conserved gene involved in this gene network and how its manipulation may influence neuronal regeneration. .

507B

Role of integrins in glial phagocytosis of apoptotic cells during *Drosophila* embryogenesis. Boris Shklyar, Flonia Levy-Adam, Estee Kurant. Department of Genetics and Developmental Biology, The Rappaport Family Institute for Research in the Medical Sciences, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.

During normal development of the central nervous system (CNS), a large number of neurons die through apoptosis and are efficiently removed by phagocytic glia, a process, which is highly conserved in evolution. The precise removal of apoptotic neurons is mediated by glial phagocytic receptors. We have previously shown that during late *Drosophila* embryogenesis, a phagocytic receptor Six Microns Under (SIMU) is required for recognition and engulfment of apoptotic cells. SIMU lacks signal transduction motifs in its short cytosolic sequence suggesting it requires a co-receptor to promote engulfment of apoptotic cells. However, the nature of this co-receptor remains unknown. Since SIMU contains an RGE motif which similarly to the RGD consensus sequence can be an integrin binding motif, we suggest integrins interact with SIMU and play a role in glial phagocytosis. Integrins are evolutionary conserved transmembrane receptors which are involved in a wide range of cellular processes such as adhesion, motility, cell survival and phagocytosis. Integrins participate in apoptotic cell clearance by macrophages and non-professional phagocytes, through direct or indirect binding to ligands on apoptotic cells and by further transducing signals into the cell to promote phagocytosis. This work focuses on genetic and molecular interactions between integrins and SIMU during engulfment of apoptotic cells. We have evaluated integrin activation in late *Drosophila* embryonic CNS, and tested physical interactions between SIMU and integrin subunits by coimmunoprecipitation. We are currently evaluating the phagocytic phenotype of RNAi knockdowns for different integrin subunits specifically in embryonic glia. These experiments will shed light on the role of integrins in glial phagocytosis of apoptotic neurons during *Drosophila* embryogenesis and contribute to better understanding of apoptotic cell clearance in the developing CNS.

508C

Ecdysone Receptor and Ultraspiracle Mediate Activation and Repression in Mushroom Body γ Neuron Pruning. Kathryn Lee, Rachael Wachter, Adam Windham, Alper Dincer, Christopher Dunne, Devin Gordon-Hamm, Elizabeth Marin. Biology Department, Bucknell University, Lewisburg, PA.

The mushroom body is a brain structure responsible for olfactory learning and memory that has become a major model for neuronal pruning and degeneration. It consists of three main classes of neurons (γ, α′/β′, α/β) projecting to five distinct axon lobes (γ, α′, β′, α, β). The γ neurons are of particular interest because they undergo developmentally regulated pruning in the early pupal stages, only to re-extend into a medial adult-specific lobe later in metamorphosis. The steroid hormone ecdysone has been shown to mediate this pruning. Ecdysone reception is mediated by heterodimers consisting of one of three isoforms of the ecdysone receptor (EcRA, EcRB1, and EcRB2) plus its co-receptor Ultraspiracle (USP). EcRB1 is expressed at high levels in the γ neurons at the onset of puparium formation. Mutations in EcRB1 have been shown to prevent γ neuron pruning, but we find that knocking down either EcRB1 or all three EcR isoforms with RNAi has no effect on pruning. Specific mutations in *ultraspiracle* have also been show to prevent γ neuron pruning, but we find the knockdown of *usp* via RNAi neither prevents nor accelerates γ neuron pruning. Furthermore the knockdown of *usp* leads to an upregulation of EcRB1 expression, suggesting that EcRB1 expression is normally repressed by USP in the absence of ecdysone. We have developed a *UAS-dicer-2; OK107-GAL4* driver strain to enhance the efficacy of RNAi knockdown in the mushroom body. We are currently using this driver to knock down *usp,* and all three*EcR* isoforms in order to rule out the possibility that gene expression was not sufficiently reduced in previous experiments that produced no effect on pruning. We are also expressing dominant negative EcR constructs in the mushroom body, one of which ($EcRB1^{W650A}$) appears to prevent γ neuron pruning, while the other ($EcRB1^{F645A}$) has no effect on pruning. These studies will help to elucidate the role of EcR/USP receptor complex in the developmental timing of neuronal remodeling.

509A

Investigating the molecular mechanisms of Crimpy-Gbb signaling at the NMJ. Kendall Hoover, Rebecca James, Heather Broihier. Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The BMP pathway is a central regulator of synaptic growth and neurotransmitter release at the Drosophila neuromuscular junction (NMJ). Interestingly, distinct pools of the BMP ligand Glass bottom boat (Gbb) control structure and function of NMJ. While presynaptic Gbb promotes neurotransmission, postsynaptic Gbb promotes synaptic growth, arguing for separable molecular cascades. However, the molecular mechanisms enabling discrimination between these pools of Gbb have remained obscure. Excitingly, our lab discovered the

neuronal transmembrane protein Crimpy (Cmpy) is essential for trafficking Gbb to the presynaptic membrane. Particularly, Cmpy sorts Gbb into dense core vesicles (DCVs) for activity-dependent release. In absence of Cmpy, Gbb is no longer associated with DCVs and is not released following activity. Moreover, a C-terminal fragment of Cmpy is secreted from presynaptic terminals upon DCV exocytosis. This co-release of Gbb and Cmpy from presynaptic terminals defines a neuronal pro-transmission signal. We are currently expanding these studies to investigate the roles of Crimpy-Gbb signaling in the regulation of synaptic vesicle behavior since both *cmpy* and *gbb*mutants exhibit defects in presynaptic vesicle cycling. Furthermore, we are identifying downstream components and assessing their individual contributions to the pro-growth and pro-neurotransmission pathways. .

510B

***Pak* (*p21-activated kinase*) mutations cause defects in brain structure and neurite-arbor morphogenesis through regulation of non-muscle myosin. Sara A Lewis**[1,2,3], Linda L Restifo[1,2,3]. 1) Neuroscience Graduate Program, University of Arizona, Tucson, AZ; 2) UA Neuroscience; 3) UA Neurology.

Drosophila Pak is the ortholog of human *p21-activated kinase 3* (*PAK3*). *PAK3* mutations cause intellectual disability with microcephaly and craniofacial defects, but little is known about the cellular neuropathology. Pak mediates Cdc42 and Rac GTPase activity in response to extracellular cues, such as adhesion and growth factors. Pak inhibits myosin function and F-actin cleavage by cofilin, thereby promoting F-actin stabilization. We used nonsense mutations from independent mutageneses to reveal that *Pak* is required for mushroom body (MB) morphogenesis during metamorphosis. *Pak*-mutant brains showed missing and misdirected α and β lobes, and became worse over time. We used primary culture to identify mechanisms of *Pak* function at the single-neuron level. Cultured *Pak*-mutant larval CNS neurons have small neurite arbors with reduced length and branching. These neurites have increased curvature with regions of excessive width, reminiscent of the filagree defect caused by fascin deficiency. Initial neurite outgrowth and branch formation appeared normal *in vitro*, but were followed by reduction in branch number, suggesting excessive branch retraction. Cytoskeletal abnormalities were present in *Pak*-mutant growth cones and neurites. The MB-lobe and neurite-arbor phenotypes map to *Pak* based on deletions and transgenic rescue. Genetic interaction studies were used to elucidate mechanisms by which *Pak* regulates neurite-arbor morphology. Pak indirectly inhibits myosin regulatory light chain (MRLC) function, thereby reducing myosin-actin interactions. Transgenic addition of non-phosphorylatable MRLC ($sqh^{A20, A21}$) suppressed the *Pak*-mutant neurite-arbor phenotype, restoring size and complexity. Genetic reductions in function of *Pak* and *flw*, encoding MRLC phosphatase, synergistically reduced neurite-arbor size. Distinct myosin isoforms can anchor polymerizing actin in growth cones, mediate retrograde actin flow, or retract branches. Together, our data support a working model whereby Pak stabilizes the nascent neurite arbor of cultured CNS neurons by inhibition of non-muscle myosin.

511C

The role of sialic acid in *Drosophila* nervous system. H. Scott[1], R. Islam[1], C. Caster[1], M. Zoran[2], V. Panin[1]. 1) Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX; 2) Department of Biology, Texas A&M University, College Station, TX.

Sialic acid is present in a variety of organisms including bacteria, insects and vertebrates highlighting its importance in many biological processes.Its role in mammalian neural development via the cell adhesion molecule NCAM is well known.However emerging evidence shows sialylation is necessary for regulating neural transmission as well.Yet the genetic and molecular mechanisms remain poorly understood given the complexity of vertebrate sialylation.We utilize *Drosophila* as a model with simplified sialylation, well-characterized neural development, and superior genetic approaches.Our lab has described the function of DSiaT, the sole sialyltransferase in *Drosophila*.DSiaT is involved in neural development and regulates neural transmission.In order to understand the regulation of sialylation in neurons, we studied the function of CMP-Sialic acid synthetase,CSAS.CSAS creates the substrate for DSiaT and functions directly upstream in the pathway.*CSAS* is expressed in the CNS throughout development suggesting a nervous system-specific function.Unlike mammalian CMP-Sia synthetases functioning in the nucleus, CSAS works in the Golgi representing an evolutionary shift in localization of a conserved protein.By employing genetic and neurobiological assays we found that *CSAS* mutants mimic *DSiaT* mutations.Similar to *DSiaT, CSAS* interacts with ion channel genes involved in neural excitability, and electrophysiology revealed that *CSAS* mutants display lowered excitability indicating a specific pathway regulating neural transmission by sialylation.We found a critical time point in development that CSAS expression was needed in order to maintain proper neural transmission.Results revealed complex genetic interactions between *DSiaT* and *CSAS*, intimating these genes may have independent functions within the nervous system separate from their role in generating sialoglycoconjugates.Our research provides the first comprehensive in vivo study of a eukaryotic CSAS, thus providing insight into the mechanisms of sialylation and its role in neural transmission likely conserved between *Drosophila* and mammals. .

512A

Expression of Sox Neuro in development of adult nervous system. Shweta Singh, John Nambu. Florida Atlantic University, Biological Sciences, 5353 Parkside Drive, Jupiter FL-33458.

Many aspects of development are controlled through the actions of specific transcription factors that regulate gene expression patterns. The mammalian *Sry* gene (sex determining Y chromosome) was discovered in 1990 and encodes a transcription factor with single high mobility group DNA binding domain. More than 20 transcription factors in humans and 8 in *Drosophila* share a related HMG domain with at least 50% identity to that of Sry. These Sox (Sry box) proteins bind to the minor groove of DNA and induce 70° to 90° bends to regulate chromatin structure and transcription initiation. There are 4 closely related members in the B group, *Dichaete (D),*

SoxNeuro (SoxN), Sox21a, and *Sox21b* that each exhibit ~90% sequence identity within the HMG domain. Interestingly in both mammals and flies, related Sox genes are often expressed in the same cells where they exhibit partially overlapping functions in the same developmental pathway. Previous study has shown that *Dichaete* play an important role in embryonic nervous system development and is expressed in several clusters of neurons in the brain, including intermingled olfactory LNs and central complex neurons strongly expressed in local interneuron of the olfactory system. However, little is known about the potential expression and functions of the related group B Sox genes in the adult brain. In particular, it is unclear if these genes may function along with *Dichaete* in controlling the development or physiology of the adult olfactory system. In order to address this issue, we will utilize a combination of genetic and cellular approaches, including the UAS/Gal4 system, RNAi, immunocytochemistry. Our data suggests SoxN is expressed in neurons and glia of the adult central brain. Interestingly, there appear to be significant overlap with the *Dichaete*-expressing cells and we are currently determining the precise identities of the *SoxN*-expressing brain cells. Furthermore, the result of this study will lead to a better understanding of Sox gene functions in both conserved and specific aspects of development.

513B

Identification of a New Regulator of Neuron Glia Interaction during Development. Diana Luong, Roni Milgrom, Jennifer Jemc. Biology, Loyola University Chicago, Chicago, IL.

Abnormalities in cell migration and cell-cell interactions can lead to severe developmental defects, disease, and death. In the nervous system, the migration and interaction of neurons and glia are critical throughout the lifetime of the organism. Glial cells provide contact mediated guidance cues for axon growth, promote electrical conductance, and maintain the blood-brain barrier via the formation of septate junctions. In the peripheral nervous system, several subpopulations of glia have been identified, including wrapping glia, which appear to function analogously to the myelin sheath found in vertebrates. Therefore, identification of genes that are critical for neuron-glia interactions in *Drosophila* can provide valuable insight into genes that may be required for neuronal ensheathment in vertebrates as well. Previous work identified a role for the novel protein Raw in ensheathment of germ cells by somatic gonadal precursor cells in the embryonic gonad. Therefore, we were interested in the possibility that Raw might perform a similar function in the nervous system. Using an RNAi based approach, we find that knockdown of *raw* in glia is lethal. Furthermore, reduced levels of *raw* in glia in the third instar peripheral nervous system results in a reduction of Repo positive nuclei along peripheral nerves. In addition, we have also seen that in the mutants the lower levels of this gene cause axonal bulging, suggesting the presence of defects in neuronal ensheathment. These results suggest that Raw plays a role in glial migration and glial ensheathment of neurons in the developing peripheral nervous system. .

514C

Self-renewal capacity of sensory organ precursor cells. Joseph Ayeni[1], Agnès Audibert[2], Pierre Fichelson[3], Martin Srayko[1], Michel Gho[2], **Shelagh Campbell**[1]. 1) Dept Biological Sci, University of Alberta, Edmonton, AB, Canada; 2) Laboratoire de Biologie du Développement (LBD-IBPS) Université Pierre et Marie Curie [UPMC] - Paris VI UPMC UMR 7622, Université Paris 06. 9, Quai St. Bernard 75005 Paris; 3) Health Interactions Admiral House, London, EC1V 9AZ.

Developmentally regulated cell cycle arrest is a fundamental feature of neurogenesis that is thought to be important for coordinating cell division with correct cell fate determination. The precise role that quiescence plays during neural development remains poorly understood, however. Moreover, growing evidence links defects in neural quiescence to neurodegenerative disease and tumour formation in humans. To study this phenomenon we examined thoracic sensory organ development, seeking to understand how the regulation of G2/M timing is coordinated with neuronal cell fate specification. Phenotypic analysis and time-lapse imaging of the thoracic microchaetae lineage showed that forcing sensory organ precursor (pI) cells to divide prematurely resulted in production of supernumerary external sensory organ cells. These supernumerary cells did not arise from defects in segregation of cell fate determinants that were previously shown to cause similar phenotypes. Instead, regulation of G2 phase quiescence ensured that neural progenitor cells did not undergo self-renewal before reception of a developmental signal that promotes neuronal differentiation. The cell cycle arrest mechanism that regulates the timing of mitosis is therefore important for synchronizing pI progenitor cell division with signals that potentiate neuronal cell fate, during development of a sensory organ lineage.

515A

Autophagy serves as a backup cell-death mechanism to ensure the removal of unwanted cells in chordotonal organ development. Adel Dunayevskyy, Adi Salzberg. Department of Genetics, Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Haifa, Israel.

Coordinated locomotion of *Drosophila* larvae depends on sensory input from stretch receptive proprioceptors named chordotonal organs. The chordotonal organs sense relative displacement of body parts and transmit this information to the central nervous system. Each abdominal hemisegment of the larva contains eight chordotonal organs, five of which are clustered together and constitute collectively the pentascolopidial organ (LCh5). Each of the five chordotonal organs within the LCh5 cluster originates from a single precursor that goes through four asymmetric cell divisions to generate five cells with different identities: a neuron, a scolopale, a cap, a ligament and a cap-attachment (CA) cell. However, whereas the LCh5 cluster contains five neurons, five scolopale cells, five ligament cells and five cap cells, it contains only two CA cells. The reason for this discrepancy and the underlying mechanism for the loss of three CA cells remained unknown. Here we show for the first time that three of the five initially developed CA cells undergo cell death during early stages of LCh5 development. We show that, normally, these cells die by apoptosis, as suggested by the presence of apoptotic cells in the immediate surroundings of the surviving CA cells and by the survival of the doomed CA cells in P35-expressing embryos. If,

however, the apoptotic pathway is blocked prior to caspase liberation, the CA cells are eliminated by another cell death mechanism, most likely autophagy. The removal of three CA cells is crucial for proper morphogenesis and function of the LCh5 organ. If cell death is inhibited and the elimination of three CA cells is thus prevented, the surviving five CA cells remain small cells and they often fail to withstand the mechanical strain imposed on them by muscle contractions and larval growth. Our finding attest to the ability of cells to switch to autophagy upon perturbation of developmental apoptotic cell death, a mechanism that can function as safeguard system to guarantee elimination of unwanted cells within a developing organ. .

516B

Alcohol addiction affects stress tolerance, movement behavior and female reproductive ability in *Drosophila melanogaster:* A possible link to dopamine synthesis pathway. Anathbandhu Chaudhuri[1], Karlson Udebuani[1], Elizabeth Caver[1], Mary , J Krotzer[1], Janis O'Donnel[2]. 1) Department of Natural Sciences, Stillman College, Tuscaloosa, AL; 2) Biological Sciences, University of Alabama, Tuscaloosa, 35487, AL.

We report here the effects of alcohol (EtOH) addiction on stress tolerance, movement behavior and reproductive performances using *Drosophila melanogaster* as a model system. To elucidate the role of dopamine pathway (DA), flies mutations in different key regulatory genes in catecholamine synthesis (DA) pathway were used for this experiment. Wild type flies have no mutations were used as control to compare the effect of EtOH with DA mutant flies. Different doses of EtOH (5, 10, 20 and 50%) were fed to the flies mixed with 5% sucrose solution. Only 5% sucrose solution was used as control. All the flies (irrespective of mutations and sex) became hyperactive upon feeding of alcohol and circle parallel to the vial with frequent fall when climbing. Also, the flies mutations in the genes involved in DA synthesis pathway showed significantly differential movement behavior and stress tolerance upon EtOH exposure. Interestingly, the female flies addicted with alcohol produced less number of offspring and alters the reproductive behavior. It was also noted that alcohol addiction could be lethal and have significant impact on female reproduction. Taken together, we hypothesized that DA homoeostasis is essential for movement and reproductive behavior and has a significant link to alcohol addiction.

517C

Ecdysone Receptor Modulates Nociception in *Drosophila melanogaster*. A. McParland, T. Follansbee, GK. Ganter. University of New England, Biddeford, ME.

The modulation of pain by steroid signaling has been the subject of some interest in mammals, but has not yet been described in insects. Differential perception of painful stimuli by male and female humans, for example, indicates a connection to gonadal steroids. Corticosteroids and the HPA axis have also been implicated in human pain perception. Furthermore, ecdysteroid signaling has been shown to play a role in regulating the pruning process, by which nociceptive dendrites are degraded in early metamorphosis. In *Drosophila*, the major if not only steroid hormone, ecdysteroid, regulates many aspects of development as well as neural processes such as learning, memory, and sexual behavior. To test the hypothesis that ecdysteroid modulates nociception, we measured avoidance responses of mutant larvae to noxious mechanical and thermal stimuli. Using mutations affecting the expression of the nuclear ecdysone receptor (EcR), its isoforms EcRA and EcRB1, and its nuclear co-receptor ultraspiracle (USP), we found significant changes in sensitivity to thermal (45°C probe) and mechanical (46mN von Frey) stimuli as compared to controls. Additionally, EcRB1 appears to play a strong role in regulating the dendritic field of class IV multi-dendritic sensory neurons, as EcRB1 mutants showed a reduction in both overall dendritic length and branching density of the primary nociceptors. These results indicate that the genetically tractable *Drosophila* system can serve as a model for the investigation of the basis of steroid modulation of pain and neuronal development.

518A

Ecdysis triggering hormone is necessary for fertilization of oocytes in *Drosophila melanogaster*. Matthew Meiselman, Michael Adams. University of California-Riverside, Riverside, CA.

Ecdysis triggering hormone (ETH) is a command peptide responsible for molt termination through initiation and scheduling of the ecdysis behavioral sequence in the fruit fly Drosophila melanogaster. Nevertheless, Inka cells, the sole source of ETH, and ETH mRNA persist through metamorphosis into adulthood, a period during which no ecdysis occurs. We have found that ETH deficient flies lay a number of eggs comparable to those with normal amounts of ETH, but that destruction of the Inka cells and ubiquitous silencing of the ETH receptor causes a large reduction in the number of embryos successfully fertilized. Several methods were used to block ETH release, including killing of the inka cells, expression of tetanus toxin or activation of the temperature sensitive *UAS-Shibire* in inka cells. While ubiquitous silencing of ETHR with the Tubulin and Ubiquitin gal4 drivers have been able to recapitulate this data, neuronal ETHR silencing has not. Rescue with agonists for both the ecdysone and juvenile hormone receptors, the two dominant regulators of oogenesis, have been unable rescue this phenotype, suggesting direct control over the process of fertilization.

519B

Differential Effects of Global Depletion of Ecdysteroid or its Receptor on Nociception. Gwendolyn Vesenka, Geoffrey Ganter. Biology Department, University of New England, Biddeford, ME.

Human studies have shown gender differences in pain perception, suggesting a role for gonadal steroids in the modulation of pain. Our lab uses *Drosophila* larvae as a model for understanding how steroid hormones are involved in nociceptive behavior. The major hormone in this class, ecdysteroid, is responsible for the regulation of numerous key pathways throughout fly development. Larvae raised on deltaERG2 mutant yeast are deficient in the sterol required for ecdysteroid synthesis. We have shown that this global

depletion of ecdysteroid results in a significant hypersensitive behavioral response to noxious mechanical and thermal stimuli. In addition, we are using RNAi tools to deplete the prothoracic gland of PTTH receptor in order to genetically decrease ecdysteroid synthesis. We also show that global hypomorphy of ecdysone receptor activity, achieved using null and temperature-sensitive alleles at the restrictive temperature during the third instar, results in animals exhibiting hyposensitive behavior to the same noxious stimuli. These contrasting results confirm the intricate and important role steroid hormones play, of which the modulation of pain nociception is only one part. We hypothesize that another neurosteroid receptor, such as the membrane-bound dopamine-ecdysone receptor or an isoform of the GABA receptor, may be involved in nociception.

520C

Suppression of *GADD45* and stress-activated protein kinase pathways reduces severity of neurological phenotypes displayed by a voltage-gated sodium channel *Drosophila* mutant,*Shudderer*. H. Chen[1], P. Landson[1], J. Kasuya[2], T. Kitamoto[1,2]. 1) Interdisciplinary Genetics Ph. D. Program, University of Iowa, Iowa City, IA; 2) Department of Anesthesia, University of Iowa, Iowa City, IA.

GADD45 (*growth arrest and DNA damage-induced 45*) plays an important role in biological response to various stressors, activating the stress-activated protein kinase pathways involving c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinase. Up-regulation of *GADD45* in the brain has been reported in several animal models of neurological disorders and it is considered as an outcome of physiological stresses caused by defects in the nervous system. *Shudderer* (*Shu*) is a *Drosophila* voltage-gated sodium channel mutant and displays neuronal hyperexcitability as well as behavioral and morphological abnormalities including seizure-like behaviors, down-turned wings and indented thorax. We found that *GADD45* expression is elevated in the head of *Shu* and that dietary modification reduces both severity of *Shu* phenotypes and *GADD45* expression. Interestingly, suppression of *GADD45* expression by means of *GADD45* hypomorphic mutations or RNAi ameliorated*Shu*'s morphological phenotypes. Moreover, cell type-specific induction of *GADD45* RNAi revealed that dopaminergic and GABAergic neurons are important for the observed phenotypic improvement. Genetic manipulation of *GADD45*-related downstream pathways, the JNK/p38 pathways, also modified *Shu*'s behavioral and morphological defects. Overall, our results indicate that a gain-of-function mutation in the voltage-gated sodium channel gene induces up-regulation of *GADD45* expression, which contributes to the manifestation of *Shu* phenotypes. It is likely that dietary modification reduces severity of *Shu* phenotypes by suppressing *GADD45* expression. Given that the fundamental biology is well conserved between flies and humans, our findings are expected to shed light on novel prevention or treatment of neurological disorders by controlling the activity of *GADD45* and stress-activated protein kinases through dietary modification. .

521A

Effects of diet on neurological phenotypes of a voltage-gated sodium channel mutant, *Shudderrer*. J Kasuya[1], P Lansdon[2], H-L Chen[2], T Kitamoto[1,2]. 1) Dept Anesthesia,; 2) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA.

Nutritional therapies have great potential to prevent or treat neurological disorders without serious side effects. Although effective nutritional therapies likely involve a dynamic interplay between metabolic networks and the central nervous system, the underlying action mechanisms still remain largely unknown. In order to take full advantage of currently available nutritional therapies for neurological disorders, and further develop novel dietary interventions based on a mechanistic hypothesis, we must have a better molecular understand of how certain diets can correct developmental and/or functional defects of the nervous system. For this purpose, it is of critical importance to establish and study suitable experimental models that are not only relevant to neurological disorders but also readily amenable to rigorous experimental approaches. *Shudderer* (*Shu*) is a dominant neurological mutant isolated more than 30 years ago and has recently been identified as an allele of the *Drosophila* voltage-gated sodium channel gene *paralytic* (*para*). We have serendipitously discovered that neurological and developmental phenotypes of *Shu*, including locomotor defects, spontaneous jerking, and abnormal wing posture, were drastically improved by dietary modification. We also found that *Shu* needs to be fed the "therapeutic" diet during the larval stage in order to receive a maximum benefit. We are currently investigating the effects of dietary modification on other neurological mutants and characterizing dietary components responsible for the rescue effects.

522B

Effects of diet on sleep of *Shudderer*, a *Drosophila* voltage-gated sodium channel mutant that displays seizure-like behaviors. Patrick Lansdon[1], Emily Petruccelli[1], Toshi Kitamoto[1,2]. 1) Interdisciplinary Ph.D. Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Anesthesia, University of Iowa, Iowa City, IA.

It is widely recognized that mutations in genes encoding voltage-gated sodium (Na_v) channels contribute to the etiology underlying various seizure disorders. Consistent with this observation in humans,*Shudderer* (*Shu*), a gain-of-function mutant for the *Drosophila* Na_v channel gene, shows seizure-like behavioral defects, including spontaneous leg jerking, twitching, and heat-induced convulsion. Intriguingly, we have recently discovered that these behavioral phenotypes are drastically suppressed by dietary modification. This finding provides an excellent opportunity to examine how diet influences neuronal activity and behaviors, potentially shedding light on the mechanisms of effective nutritional therapies. In this study we investigated whether dietary modification could also improve abnormal sleep behavior in *Shu* mutants. Sleep is an evolutionarily conserved, essential behavior, which requires higher-order brain function involving elaborate interactions of multiple neuronal circuits. While seizure disorders are often comorbid with disturbances in sleep, seizure-prone, hyperexcitable mutants in *Drosophila* such as *Shaker, Hyperkinetic,* and *quiver/sleepless* display various sleep abnormalities. We found that *Shu* flies also exhibited aberrant sleep patterns – decreased total nighttime sleep with a decrease in sleep bout duration and an increase in sleep bout number as compared to controls. Further, we demonstrated the fragmented sleep phenotype of *Shu* was improved by dietary modification. Currently, we are determining the effect of diet on

homeostatic regulation of sleep in *Shu*. This and future studies are expected to help us better understand the intricate interplay among epilepsy, sleep and nutritional therapies.

523C

Molecular characterization of noxious cold detection in *Drosophila* larvae. Benjamin Williamson[1], Harold Burke[1], Kevin Armengol[2], Daniel Cox[2], Susan Halsell[1]. 1) Biology, James Madison University, Harrisonburg, VA; 2) Neuroscience Institute, Georgia State University, Atlanta, GA.

The perception of pain (nociception) is a genetically conserved process in animals. Pain continues to be a major health concern and current treatments prove insufficient, especially in regards to chronic pain. Greater understanding of the molecular processes underlying pain sensation could lead to new and more effective treatments. The aim of this study is to investigate the molecular mechanisms of cold nociception in *Drosophila melanogaster*. A specific subset of peripheral sensory neurons (Class III dendritic arborization (da)), are implicated in *Drosophila* larvae's response to noxious cold. Previous literature has implicated a variety of ion channel families, including TRP and degenerin/epithelial sodium channels (DEG/ENaC) subfamiliy members, in mediating sensory responses to noxious heat and mechanosensation, including mechanosensory nociception. Though much is known about noxious mechanical and heat nociception in *Drosophila*, little is known regarding the molecular components mediating cold nociception. Here we focus on characterization of *Drosophila* DEG/ENaC family members as potential regulators of noxious cold-evoked sensory behavior. A novel behavioral assay, coupled with functional optogenetic studies and *in vivo* RNAi, has been utilized to investigate the role of select *pickpocket* (*ppk*) family members which we have identified as exhibiting significant enrichment in class III da neurons. Our analyses reveal that a subset of these *ppk* family genes are required for noxious cold detection in larvae. These studies provide novel mechanistic insight into the molecular underpinnings of cold-evoked behavioral responses and demonstrate a previously uncharacterized function for DEG/ENaC molecules in cold nociception. .

524A

Neuronal insulin signaling negatively regulates SV release in response to high protein diet. Rebekah Mahoney, Jorge Azpurua, Benjamin Eaton. Physiology, UTHSCSA, San Antonio, TX.

Obesity within the US population has reached epidemic levels within recent times resulting in a significant increase in the prevalence of type II diabetes mellitus. Studies have shown that individuals with diabetes have significantly reduced cognitive function and an increased risk for age related dementias, such as Alzheimer's disease, in comparison to their age-matched controls. Thus, it has become clear that there is an important link between poor nutrition, diabetes and brain function. We have developed a novel synapse model system in adult Drosophila to investigate the effects of diet on synapse function. Using this system, we have previously shown that a high calorie diet can dramatically reduce quantal content at the Drosophila CM9 NMJ. We have now shown that the effects of diet on SV release are due to insulin signaling within the motor neuron. Our data supports that insulin signaling through FOXO, but not mTOR, reduces the levels of eIF-4e binding protein (4eBP) resulting in a reduced quantal content. Because 4eBP is a negative regulator of eif4e-dependent translation our data supports that insulin signaling promotes the translation of a negative regulator of presynaptic release. Furthermore, it was observed that *staufen* mutants failed to reduce release when shifted to high protein diets supporting that this translational event is occurring within the nerve terminal. These data would provide a novel mechanism for the effects of insulin signaling on presynaptic release and could potentially provide new targets for treating the cognitive deficits found in individuals with age related dementias.

525B

GLUTAMATE CLEARANCE BY ASTROCYTE-LIKE GLIA REGULATES NEURONAL EXCITABILITY AND SYNAPSE GROWTH IN *DROSOPHILA* LARVAL MOTONEURONS. Jhan-Jie Peng[1,2], Shu-Hui Lin[1], Tzu-Li Yen[1], Chi-Kuang Yao[1,2]. 1) NPAS and Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.

Activity-dependent synapse growth is central to learning and memory. Clearance of glutamate, the main excitatory neurotransmitter in the vertebrate CNS, from synaptic cleft via astrocyte-expressed EAAT transporters is crucial to fine-tune the physiological level of neuronal activity. Yet, the role astrocyte-regulated neuronal activity in synapse growth has not been demonstrated. Importantly, EAATs are downregulated in patients suffering from epilepsy, stroke or many of common neurodegenerative diseases. However, how excitotoxicity is involved in these disorders is not fully understood. In present work, we used glutamatergic *Drosophila* larval motoneurons as the model system to address it. *Drosophila* excitatory amino acid transporter (dEAAT1) is the sole high-affinity glutamate transporter and highly expressed in astrocyte-like glia in the fly CNS but not at the larval neuromuscular junction (NMJ). Our GRASP assay visualized that the processes of astrocytes, which are enriched with dEAAT, closely associate with glutamatergic synapses in the ventral nerve cord. In a screen for mutations affecting NMJ bouton growth, we identified a new hypomorphic mutant allele of dEAAT1. The mutant larvae displayed uncoordinated locomotion. Through Ca^{2+} imaging and muscle intracellular recording, we found that mutant larval motoneurons obtain excess premotor input. Interestingly, loss of *deaat1* NMJs significantly expand when compared to control and astrocyte specific-rescued animals. Attenuation of neuronal activity rescued NMJ phenotype, suggesting that astrocyte-expressed dEAAT1 normally downregulates neuronal activity and thereby controls synaptic bouton growth. Moreover, with genetic analysis and immunostaining, our data strongly suggest that activated ROS-triggered JNK pathway in both neurons and muscles promotes NMJ overextension in *deaat1* mutants. Hence, our work provides a mechanistic link between astrocyte-mediated glutamate uptake and synapse growth. In addition, the fly larval motoneuron circuit would be a good model system to understand cellular and systematic roles of excitotoxicity in diseases.

526C

The role of ER morphology in the control of synaptic transmission and locomotor behavior. James Summerville, Joseph Faust, Miguel Betancourt, Joseph Formella, James McNew, Michael Stern. BioSciences, Rice University, Houston, TX.

The Hereditary Spastic Paraplegias (HSPs) comprise a diverse set of genetic diseases caused by mutations in any of almost 50 genes, named *SPG1* to *SPG48*. Age-dependent corticospinal axon degeneration, spasticity, and weakening of the lower limbs represent prominent HSP clinical features. Two genes implicated in HSPs
encode proteins that regulate ER morphology. In particular, *atlastin* (responsible for the HSP disease SPG3A) encodes a GTPase that cata lyzes ER fusion, whereas *Reticulon* (responsible for the HSP disease SPG12) encodes an integral ER membrane protein that, by providing curvature, is required for ER tube formation. These observations suggest that ER morphology plays a crucial role in development of the neuronal dysfunction present in HSPs. We are currently using cell biology, electrophysiology, and behavioral assays to elucidate mechanisms by which these proteins regulate ER morphology, synaptic transmission, and adult behavior. Using a novel fluorescent ER marker, we found that in wildtype 3rd instar larvae, the ER exists within the larger motor neuron synaptic boutons as a network of tubules that resembles a "basket" structure underlying the plasma membrane. However, the ER is fragmented in atl^2 null mutants and present in large punctae in $Rtnl1^1$ mutants. We have also observed that both atl^2 and $Rtnl1^1$ mutations decrease transmitter release from larval motor neurons, particularly at the lowest bath [Ca^{2+}]. Using cell specific RNAi we found that *atl* controls transmitter release from motor neurons, whereas *Rtnl1* controls transmitter release from both neurons and muscles. Finally we found that RNAi knockdown of *atl* confers age-dependent locomotor deficits, whereas $Rtnl1^1$ confers age-dependent sensitivity to mechanical vibration. These results demonstrate a critical role for proper ER morphology in neuronal function and identifiy mechanisitc links between ER morphology, neuronal function, and adult behavior. We anticipate these studies will provide information essential for a more complete understanding of altered neuronal biology underlying pathologies in HSPs. .

527A

Ca^{2+} influxes via Flower facilitate Clathrin-mediated and bulk endocytosis at the *Drosophila* neuromuscular junction. Chi-Kuang Yao[1,2,3], Shu-Hui Lin[1], Jing-Ming Chen[1], Tzu-Li Yen[1], Yu-Tzu Liu[1]. 1) Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 2) NPAS, Academia Sinica, Taipei, Taiwan; 3) Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.

Chemical neurotransmitter release is crucial for neuronal communication and accompanied with the fusion of synaptic vesicles to the presynaptic plasma membrane. Fused vesicle membrane is subsequently retrieved by fast, efficient endocytosis in order to maintain a proper size of the vesicle pool. Hence, there is a mechanism by which exocytosis is tightly coupled to endocytosis. We previously reported that a synaptic vesicle-localized Ca^{2+} channel Flower (Fwe) distributes to the endocytic zone upon vesicle fusion and in turn promotes Clathrin-mediated endocytosis at the *Drosophila* neuromuscular junction. We therefore proposed that the plasma membrane-bound Fwe likely triggers Ca^{2+} influxes to facilitate endocytosis and thereby links exocytosis with endocytosis. Our recent work revealed that a very low level (~ 3%) of the Fwe protein is indeed sufficient to support endocytosis, analyzed by styryl FM1-43 dye uptake, electrophysiology and transmission electron microscopy. Furthermore, the Ca^{2+} channel activity of Fwe is important under this condition. These results suggest that endocytosis can normally occur at synapses with a wide range of the Flower levels. This is quite consistent with early work in lamprey reticulospinal synapses in which very low extracellular Ca^{2+} levels could restart substantial endocytosis when endocytosis was arrested by removing extracellular Ca^{2+}. Intriguingly, we further found that Fwe-triggered Ca^{2+} influx participates in the formation of activity-dependent bulk endocytosis induced by either high K^+ stimulation or blockage of Clathrin activity. Taken together, the Ca^{2+} channel Flower functions as an important regulator for coupling exocytosis to different forms of endocytosis.

528B

Unable to forget in mutants of homologs of autism susceptibility genes in *Drosophila*. Tao Dong[1], Jing He[1], Shiqing Wang[1], Lianzhang Wang[1], Yi Zhong[1,2]. 1) School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

To investigate whether common neurobiological mechanisms underlie effects of different autism susceptibility genes, we performed assays of immediate memory and reversal-learning based cognitive flexibility on effects of homologs of multiple such genes in *Drosophila*. The well-established Pavlovian conditioning paradigm is used to measure learning ability and its reversal-learning paradigm for cognitive flexibility. We found that although showing a mild learning reduction, all mutants of multiple genes examined displayed a profound impairment in the reversal learning. In other words, these genes affect mainly cognitive flexibility, instead of learning. To determine mechanisms underlying such behavioral alterations, we focused on effects of the *fragile X mental retardation 1* (*Fmr1*) gene that encodes a RNA-binding protein. Our study demonstrated that impaired reversal learning was resulted from an inability in activating Rac1-dependent forgetting, leading to being unable to forget. This phenotype could be partially rescued by over-expression of constitutively active Rac1. Such results lead to discussion of a hypothesis as that forgetting is the converging site for autism susceptibility genes.

529C

Importin limits long-term memory consolidation in Drosophila. Qian Li[1], Xuchen Zhang[1], Xitong Liang[1], Wantong Hu[1], Fang Zhang[1], Lianzhang Wang[1], Yi Zhong[1,2]. 1) School of Life Sciences, Tsinghua University, Beijing 100084, PR China; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

It is believed that importin-mediated nuclear transport is important for long-term synaptic plasticity from invertebrates to mammals. Surprisingly, little is known whether importins participate in long-term memory formation. Here we report that an importin can bi-directionally regulate aversive long-term memory strength in Drosophila. We found acutely down-regulation of the importin during consolidation led to LTM defect, while up-regulation led to LTM enhancement. Moreover, this change on LTM strength was confined in mushroom body neurons. Thus, our results demonstrate a critical role of importins in memory consolidation.

530A

A dopamine-modulated neural circuit regulating taste memory in *Drosophila*. P. Masek[1], K. Worden[1], Y. Aso[2], G Rubin[2], A. Keene[1]. 1) Biology Department, University of Nevada Reno, Reno, NV; 2) 2. Janelia Farms Research Campus, 19700 Helix Drive, Ashburn, VA 20147.

We have developed a single-fly gustatory learning assay to functionally interrogate the neural circuitry encoding taste memories. Here, we screen a novel collection of Split-GAL4 lines that precisely label small populations of neurons associated with fly memory center - the mushroom bodies (MB). Genetic silencing of PPL1 dopamine neurons disrupts conditioned, but not naïve, feeding behavior, suggesting these neurons are selectively involved in the conditioned taste response. We identify a PPL1 subpopulation that innervates the mushroom body α lobe and is essential for aversive taste memory. Optogenetic activation of dopamine subclusters containing MB-V1 neurons during training alone induces memory, indicating these neurons are sufficient for the reinforcing properties of bitter tastant to the mushroom bodies. Silencing of the intrinsic mushroom body neurons, or the output neurons that receive input from the mushroom body α2 vertical lobes, disrupts taste conditioning suggesting these neurons are downstream of the MB-V1 dopamine neurons that are required for taste memory. Unlike dopamine neurons and intrinsic mushroom body neurons, thermogenetic manipulation of the α2 output neurons alters naïve feeding response, revealing that these neurons set the threshold of response to appetitive tastants. Taken together, these findings detail a neural mechanism underlying the formation of taste memory and provide a functional model for dopamine-dependent plasticity in *Drosophila*.

531B

Effect of hsp83 on *Drosophila melanogaster* courtship behavior. Ekaterina Nikitina[1,2], Marianna Shabanova[2], Elena Savvateeva-Popova[1]. 1) neurogenetics, Pavlov Institute of Physiology RAS, St-Petersburg, Russian Federation; 2) Herzen State Pedagogical University, St-Petersburg, Russian Federation.

Heat shock proteins (HSPs) termed as molecular chaperones are the evolutionarily conserved and essential proteins that play a key role in cell survival through cytoprotective mechanisms. The induction of the HSPs activity is also of potential benefit when structural and functional preservation of proteins may enhance cell survival upon neurodegeneration, trauma, stroke and cardiovascular disease. hsp83 is actin-binding protein which controls conformational regulation of many signal proteins, cell cycle and cell transition. Conditioned courtship suppression paradigm was used to asses learning acquisition and memory formation in transgenic *Drosophila* strain with defective hsp83 synthesis. Learning acquisition and 3-h (intermediate) memory formation appeared to be normal in intact control. However, a failure of 3-h memory formation was observed following heat shock, thereby indicating an important role of hsp83.

532C

The Genetic Basis of Learning and Thermotolerance in *Drosophila Melanogaster*. Anna M Perinchery, James Mrkvicka, Elizabeth King, Troy Zars. University of Missouri, Columbia, MO.

In a variable temperature world, there is adaptive value in the ability of an animal to learn from, or tolerate, temperature extremes. This is especially true for small poikilotherms, like *Drosophila*, where body temperature reflects ambient temperature very quickly. Previous results show that fruit flies can use memories to make decisions. Place memory experiments have been important in exploring the fundamental principles of the formation of memory. Certain genes have been found as candidates for determining place memory though experiments with genetic mutants. However, next to nothing is known about how naturally occurring variation influences place memory. To explore this we used a unique and powerful genetic mapping population, the *Drosophila* Synthetic Population Resource (DSPR). The DSPR is a panel of more than 1500 recombinant inbred lines (RIL) that have been generated from an eight-way, 50-generation cross. We tested female fruit flies from the DSPR for learning ability and thermotolerance using a Heat-Box. The position of a single fly is monitored within a chamber by this device; when a fly crosses the midline, the Heat-Box heats up. Highly aversive temperatures test how well fruit flies learn to stay on one side of the chamber. The same flies are also tested for tolerance to heat. From here, we mapped QTLs for learning and tolerance. Lastly, we examined the potential relationship between heat tolerance and learning ability.

533A

Neural pathways routing early memory decay in *Drosophila*. Yichun Shuai[1], Areekul Hirokawa[1], Yulian Ai[1], Wanhe Li[2], Yi Zhong[1]. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Laboratory of Genetics, Rockefeller University, New York, NY.

The fruit fly olfactory aversive memory is susceptible to rapid forgetting shortly after training. The mushroom body (MB) has been implicated as an important brain locus for both olfactory memory formation and forgetting. It remains a paradox how the MB network is divided or shared between these two antagonizing functions. By behaviorally screening Gal4 drivers of MB extrinsic neurons (MBENs), we uncovered two groups of memory-constraining MBENs. The inactivation of them significantly slows down early memory decay, while the brief activation of them after training reduces memory. Their blockade does not affect learning or reversal learning, suggesting that

these MBENs participate specifically in forgetting during time-dependent decay. These two groups of neurons, were genetically traced precisely to a dozen of dopaminergic neurons (PAM-β'1) and a single glutamatergic neuron (MBON-γ4>γ1γ2). They intersect with the MB β' and γ lobe respectively and bear connection within themselves. We propose that a forgetting micro-circuit embedded in the MB network guides early labile memory towards destruction during time-dependent decay.

534B

A new method for reliable scoring of olfactory preferences and learning in *Drosophila melanogaster*. Elisabetta Versace[1,2], Julia Reisenberger[1], Christian Schlötterer[1]. 1) Institut für Populationsgenetik, Vetmeduni Vienna, Wien, Austria; 2) Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy.

In the Evolve and Resequence method (E&R), experimental evolution and genomics are combined to investigate evolutionary dynamics. This approach requires many replicates with large population sizes (Schlötterer et al. 2014), which imposes severe restrictions on the analysis of behavioral phenotypes. Aiming to use E&R for investigating the evolution of behavior in fruit flies, we have developed a simple and effective method to assess spontaneous olfactory preferences and learning in large samples of fruit flies. Compared to previous methods (e.g. Mery and Kawecki 2002; Quinn et al., 1974) the new procedure reduces the environmental noise and allows for the analysis of large population samples. We tested our method on (a) a large wild-caught population and (b) 11 isofemale lines of *Drosophila melanogaster*. Consistent with previous results we show that flies have a spontaneous preference for orange when compared apple odor (see also Dweck et al. 2013). Furthermore, we find genetic differences in the olfactory learning with relatively high heritability. We propose that this new method provides an excellent tool for E&R or GWAS studies on olfactory preferences and learning.

535C

Evidence for PKA Dependent Regulation of Tomosyn, a Syntaxin Binding Protein. Sarah J Zinn[1], Szi-Chieh Yu[1], Martin Schwärzel[2], Carolin Wichmann[3], David E Featherstone[1], Janet E Richmond[1]. 1) Biological Sciences, University of Illinois, Chicago, Chicago, , IL; 2) Institute for Biology/Genetics, Free University Berlin D-14195 Berlin, Germany; 3) Department of Otolaryngology, University of Göttingen, 37075 Göttingen,Germany.

Tomosyn, a syntaxin binding protein, has been postulated to negatively regulate synaptic vesicle fusion by forming nonfusogenic complexes with the plasma membrane SNAREs, syntaxin and SNAP-25, thereby inhibiting SNARE complex assembly. Using RNAi knockdown we recently demonstrated that *Drosophila* tomosyn not only inhibits synaptic transmission but also disrupts PKA-dependent aversive olfactory learning in flies. Biochemical evidence indicates that vertebrate tomosyn is a direct PKA-target. Phosphorylation reduces the ability of tomosyn to inhibit fusogenic SNARE complex assembly by lowering its syntaxin binding affinity. The possibility that tomosyn is a potentially important PKA-target *in vivo* is supported by the following observations: 1) Acute activation of cAMP phenocopies the tomosyn loss-of-function phenotype and manifests as increased synaptic vesicle docking and enhanced release. 2) cAMP activation results in the translocation of tomosyn away from the plasma membrane and 3) cAMP activation combined with tomosyn RNAi shows no additivity, suggesting they act in the same pathway. To definitively establish that the phosphorylation of tomosyn accounts for these cAMP synaptic effects we have generated a tagged tomosyn construct using CRISPR/Cas9 for pull down and subsequent kinase activity assays to determine the most probable PKA binding site. Based on both bioinformatics and molecular evidence potential phosphomimetic and non-phosphorylatable tomosyn fly strains will be generated using CRISPR/Cas9 and will be the subject of electrophysiological and immunohistochemical analyses.

536A

Study of the courtship behavior in some sibling species of the *Drosophila virilis* group. Elena G. Belkina[1], Oleg E. Lazebny[1], Varvara Yu. Vedenina[2]. 1) Russian Academy of Sciences, Koltzov INSTITUTE of DEVELOPMENTAL BIOLOGY, Moscow, Russian Federation; 2) Russian Academy of Sciences, Kharkevich Institute for Information Transmission Problems, Moscow, Russian Federation.

The courtship rituals of *Drosophila* include an exchange of several signals with different modalities between sexes. Courtship behavior of *Drosophila* males represents sequential stereotypical elements such as orienting towards a female, touching her with their foreleg tarsi, wing vibration, circling around the female, and licking her abdomen. Both sexes receive chemical and tactile cues from each other when males touching and licking females. In particular, when touching with his fore legs, which tarsi have contact chemical receptors, the male is suggested to recognize the female epicuticular hydrocarbons. In the course of wing vibration, the males produce acoustic and sometimes visual cues; when circling around the female, the males produce visual stimuli. Signals of same modalities may crucially differ between closely related species. Courtship behavior is studied in four closely related *Drosophila* species: *D. virilis*, *D. lummei*, *D. a. americana* and *D. littoralis*. Using a videocomputing approach, we compare behavior in intact and chemically and surgically treated males courting conspecific and heterospecific females. Removing particular parts of the flies, namely, wings and tarsi in males and arista and antennae in females or treating males' tarsi and females' antennae with zinc sulphate solution we block specific receptors and thus affect the patterns of courtship behavior. We find touching and licking to be the most prolonged courtship elements in all species studied despite the fact were flies treated or untreated. Touching and licking are typically proceeding together usually accompanied by wing vibration. So far, we found only minor interspecific variations in courtship rituals. Heterospecific courtships in *D. virilis* and *D. a. americana* are almost active as conspecific ones with *D. a. americana* males being significantly more active than males of the opposite species. The project is in progress now.

537B

Effect of Central Brain Histamine Deficiency on Courtship Behavior in *Drosophila melanogaster*. Tina Daniels[1], Laura Schroeder[1], Martin Burg[1,2]. 1) Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Cell & Molecular Biology, Grand Valley State University, Allendale, MI.

Histamine is a biogenic amine that has been shown to be necessary for a number of functions including vision, grooming, and temperature preference. Mutations in the *Hdc* gene, which disrupts HDC function, have been used to identify the effects of histamine deficiency on these behaviors. Histamine has been localized to peripheral sensory receptor cells (photoreceptor and mechanosensory receptor cells) and a small number of central brain neurons. Thus far, it has not been possible to separate the function of the histaminergic neurons in the CNS from that of the PNS, as *Hdc* mutations eliminate or severely reduce histamine levels in all cells through reducing *Hdc* transcript levels. Previously, we have characterized an *Hdc* transgene P[$gHdc^{+};w^{+}$] in an Hdc^{JK910} mutant background that rescues theHdc^{JK910} mutant phenotype completely, restoring histamine in all cells and developmental stages. A deletion in the 5′ noncoding region of the P[$gHdc^{+};w^{+}$] transgene was made (P[$gHdc^{\Delta 38};w^{+}$]) that has been shown to prevent *Hdc* expression in adult central brain neurons when placed in a Hdc^{JK910} mutant background. We have used this $gHdc^{\Delta 38}$ transgene deletion mutation to determine whether histamine deficiency in the central brain could disrupt a complex behavior, such as courtship. Virgin male and female flies of the same (homotypic) or different (heterotypic) genotypes were introduced into a small chamber to observe courtship behavior, and the time after introduction at which various steps of courtship were exhibited was recorded. Results indicate that a total lack of histamine typical ofHdc^{JK910} mutants has a profound effect on the ability of flies to exhibit a normal courtship behavioral repertoire. Results from both homotypic and heterotypic courtship assays indicate that both male and females with only a CNS histamine deficiency appear to be disrupted in separate aspects of courtship. This result demonstrates the use of the transgenic g$Hdc^{\Delta 38}$ deficiency in addressing the question concerning central vs. peripheral histamine function and its effects on a complex behavior, such as courtship. .

538C

Mating Success is Influenced by Multiple Signal Modalities in *Drosophila saltans*. Jennifer M. Gleason, Kaila Colyott, Cynthia Odu. Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

In *Drosophila* courtship, multiple sensory channels are used by the male and female to achieve mating success, but the relative importance of each can differ for the sexes. Vision is used for both tracking the other individual and for receiving signals. Courtship song, an auditory signal is produced by the male and perceived by the female. Chemical signals, such as pheromones, are also exchanged between the sexes. Tactile signals may also play a role because the sexes are in physical contact in advance of mating. Changes in sensory modality use within species groups can potentially contribute to speciation through changes in courtship signals and their reception. Within the *Drosophila saltans* species group, courtship song has evolved quickly, leading us to ask what role audition and the other sensory modalities play in copulation success. To determine how the senses influence mating success in *D. saltans sensu stricto,* we compared mating success and courtship timing variables among individuals with ablated and intact senses. As predicted by the variability of courtship song in the group, the absence of the auditory signal reduces mating success if either the male cannot produce wing song or the female cannot hear it. However, audition is not the only sensory modality influencing mating success. Blindness, caused by covering the eyes, decreases male, but not female, mating success. Because testing olfaction requires removal of the antennae, which also removes hearing through the aristae, isolating the individual effects of olfaction ablation is not possible; the double effect of removing the perception of auditory and olfactory signals for males eliminates all mating success. The absence of gustation through foretarsi significantly decreases the probability that males will initiate courtship. We also found a new courtship component: midtarsi tapping. When females lack midtarsi, copulation success decreases. We hypothesize that females may signal receptiveness in this species by tapping the male with her midleg. Overall, we find that males and females rely on different sensory channels for the conveyance of signals and that multiple sensory modalities influence mating success.

539A

Expression of the Female Specific Transformer Protein using the *Trapped in endoderm-1* Promoter Decreases Wing Song Latency. P. Luu, D. Tran, R. French. Biological Sciences, San Jose State University, San Jose, CA.

In a previous functional dissection using the GAL4 system to inhibit the male specific Fruitless protein, Meissner et al. identified the strain 9-210 as having a short latency to wing song during courtship. We found that 9-210 carried three X-linked GAL4 insertions in the gene, near *CG42343*, *folded gastrulation* (*fog*), and *Trapped in endoderm 1* (*Tre1*). We have identified the Tre1 insertion (*Tre1*-GAL4) as the major contributor to the shorter wing song latency when used to drive expression of the female specific transformer (TraF). In order to localize the effect, we have used immunofluorescence imaging on adult flies expressing GFP with the *Tre1*-GAL4 driver and found expression primarily in the peripheral nervous system. We are now investigating the roles of these neurons in the short courtship latency time, as well as characterizing any fitness deficits resulting from being too quick to initiate courtship. Finally, because *Tre1*encodes a G-protein coupled receptor (GPCR) with high homology to gustatory receptors, we are investigating the role of *Tre1* in courtship initiation, and how it contributes to the involvement of gustatory cues in courtship. The results of these investigations will be presented.

540B

The transcription factor *dati* is required for female courtship acceptance. Joseph Schinaman[1], Rachel Giesey[1], Claudia Mizutani[1], Tamas Lukacsovich[2], Rui Sousa-Neves[1]. 1) Case Western Reserve University, Cleveland, OH; 2) University of California, Irvine, Irvine,

California.

Courtship is a behavior in which members of one gender advertise to the other their species identity and overall fitness. In Drosophila, the male's courtship display provides a complex array of auditory, visual and olfactory information to the courted female, which she must decode and compare in order to generate the decision to accept or reject the potential mate. However, relatively little is known about the neural circuitry underlying this ability to integrate disparate types of information into a discrete behavioral output. In this work, we show that females mutant for the Krüppel-like transcription factor *datilógrafo* (*dati*) are incapable of accepting males, despite eliciting normal courtship from them. A clonal analysis of female *Drosophila* bearing genetic lesions of *dati* throughout the brain revealed three regions where this transcription factor is required to facilitate normal acceptance behavior: the projection neurons of the antennal lobe, the lateral horn, and the posterior superior lateral protocerebrum. Further narrowing down this circuit, an RNA interference screen showed that *dati* is required in cholinergic neurons specifically to generate normal acceptance, ultimately implicating only around 15 neurons in the antennal lobe and lateral horn, and as few as 4 in the protocerebrum. In whole, this work highlights indispensable and tractably-sized areas of the overall courtship acceptance circuit, and, in showing the need for a convergence of excitatory neurons in areas of sensory integration, provides evidence for a stimuli-summation method of courtship acceptance.

541C

Increased dietary macronutrients affect *Drosophila melanogaster* reproductive behavior. Janna Schultzhaus, Ginger Carney. Biology, Texas A&M, College Station, TX.

Dietary composition affects female *D. melanogaster* fecundity, indicating that diet can affect fitness. If male and female mate preference is based on perceived fitness of potential mates, then animals may possess a mechanism for judging mate quality based on phenotypic characters. We hypothesize that changes in mate quality brought about by varying diet will influence mate preference. To test whether macronutrient content alters mating behavior and whether changes in mate preference correlate with dietary effects on fecundity, we raised flies on a base diet or on diets with increased sugar, protein, or fat content. We video recorded mating behaviors between and among groups provided with different diets and quantified male courtship behaviors, including courtship latency, courtship index, copulation attempts, and copulation duration, as a measure of male mate preference. Mating latency was used as a measure of female mate preference, and total eggs laid for five days post-copulation were counted as a measure of female fecundity. We found that high fat and high protein diets had significant effects on all reproductive parameters measured, while the high sugar diet affected only the proportion of time that males spent courting females. As expected, high fat females were both less fecund and less preferred by base males, and high sugar females showed no changes in either fecundity or in attractiveness to base males. Unexpectedly, although our results indicate that high protein females were more fecund, their attractiveness to base males was unchanged. Females that mated with high protein males were less fecund, but females showed no change in mate preference towards high protein males. In conclusion, increased macronutrient content affected many aspects of mating behavior, but mate preference was not always correlated with fecundity as was expected.

542A

Complex social behavior in Drosophila: ethanol and natural genetic variation in courtship and aggression. Sarah Signor[1], Mohammad Abbasi[1], Brad Foley[1], Paul Marjoram[1], Sergey Nuzhdin[1], Lauren McIntyre[2]. 1) Department of Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL.

D. melanogaster is a key model system for the genetics of complex traits (e.g. behavior) and complex diseases (e.g. neurological diseases). Complex traits are the product of many interacting alleles with context dependence effects. Therefore to thoroughly study a complex behavioral phenotype requires its examination in different genetic and social contexts, in addition to their interactions with environment. Here, we study the social effects of pharmacologically relevant concentrations of ethanol, because *Drosophila* is an accepted model species for the study of its abuse. Each behavioral assay contained two genetically identical males and one female, with variation in complex behaviors quantified using high throughput assays and automatic tracking of the individual flies. The automatic tracking methods use vision and machine learning and do not interfere with social signaling by the flies, a marked improvement on previous methods. Despite the males being genetically identical there was intense competition for access to the female ($P<0.0001$). Overall alcohol increased the activity of the flies ($P<0.0001$), but also surprisingly pacified them. That is to say that alcohol had dramatic effects on reducing overall aggression ($P<0.0001$), with stronger effects in some genotypes and weaker in others ($P<0.0001$). Likewise, the frequency of male courtship towards females was dramatically decreased in ethanol treatments ($P<0.0001$). Interestingly, while all participants were experiencing ethanol exposure this did not decrease the 'choosiness' of the females, rather ethanol reduced the mating rate to almost zero. We have also found some of the best evidence to date for indirect genetic effects, as female behaviors changed as a function of male genotype ($P<.0006$). This model of fly sociality under the influence will clarify the aspects of developing a complex disease in genotype specific way.

543B

Expression of a mutant form of human CHMP2B in the *Drosophila* CNS is capable of disrupting circadian rhythms. Christopher Krasniak, Joshua Kavaler, Tariq Ahmad. Department of Biology, Colby College, Waterville, ME.

Frontotemporal dementia (FTD) is the second most common age correlated dementia. One protein implicated in FTD is CHMP2B, which when mutated can cause disruptions in the functioning of an ESCRT-III component. ESCRT proteins are required for the forming

of multivesicular bodies, an important component of the endosomal-lysosomal pathway. Disruptions in this pathway are linked to numerous diseases including Huntington's disease, amyotropic lateral sclerosis, and FTD. FTD, as well as many other neurodegenerative diseases, has been shown to disrupt the sleep-wake cycles of patients dictated by circadian rhythms. Here we show that misexpression of a mutant form of CHMP2B in the *Drosophila* nervous system using the GAL4-UAS system leads to disruption of circadian rhythms. We have also attempted to determine if the disruption is due to expression of CHMP2B in pacemaker cells. Specific results will be further discussed.

544C

RNA-Seq Reveals Age-Induced Changes in Rhythmicity in the *Drosophila* Transcriptome. Rachael Kuintzle[1], Eileen Chow[2], Jadwiga Giebultowicz[2], David Hendrix[1,3]. 1) Biochemistry & Biophysics, Oregon State University, Corvallis, OR; 2) Integrative Biology, Oregon State University, Corvallis, OR; 3) School of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR.

A robust circadian clock is well known to be an integral part of healthy aging. Senescent flies and mammals exhibit irregular behavioral and sleep rhythms, which are associated with neurodegeneration and increased susceptibility to oxidative stress. In flies, these phenotypes are exacerbated in clock gene mutants, suggesting that the ability to maintain strong clock function may slow down aging and extend healthspan. However, the molecular basis for age-related circadian dysregulation is unknown. To investigate this, we performed RNA sequencing (RNA-Seq) and compared the temporal expression profiles of circadian transcripts in young and old *Drosophila melanogaster*. Our hypothesis that aging of the circadian clock may lead to gene hypofunction or hyperfunction was supported by many instances of altered rhythms in regulatory genes and corresponding changes in expression of their target genes. In addition, we discovered several co-expressed circadian gene clusters with associated long noncoding RNAs exhibiting coordinated, age-induced transcriptional changes. Our data also revealed a potential novel, multi-exonic, age-activated noncoding gene with rhythmic expression. This study demonstrates extensive and diverse effects of aging on rhythmic RNA levels and provides a genome-wide map of isoform-resolution expression in senescent *Drosophila*.

545A

Analyses of alcohol induced behavior in *Drosophila period* mutants. Jennifer Liao, Tariq Ahmad. Colby College, Waterville, ME.

Circadian rhythms maintain an organism's daily sleep-wake cycle by conserved regulatory pathways, inducing profound effects on changes in metabolic activity. *Drosophila* circadian period is maintained in a 24-hour cycle with peaks of activity at dawn and dusk. Ethanol exposure causes disruptions in a variety of physiological processes including circadian rhythms. We hypothesized that defects in circadian rhythm might lead to altered behavioral responses to ethanol and to disruptions in ethanol metabolism. To investigate this hypothesis, we used Drosophila strains bearing mutations in the *period* gene that result in circadian periods which are arrhythmic (*per0*), longer (*perL*), or shorter (*perS*) compared to wild type rhythms. We analyzed ethanol metabolism by measuring alcohol dehydrogenase (Adh) activity, an enzyme that converts alcohol to aldehyde, and characterized behavioral responses to ethanol exposure by measuring sedation time, recovery from sedation, and tolerance after repeated exposure. This study will contribute to the understanding of ethanol exposure on circadian rhythm modulations in *Drosophila*, which may facilitate the explanation of ethanol intoxication consequences on human circadian changes.

546B

Investigating the roles of glial cells in supporting circadian function and healthy aging. Dani Long, Jadwiga Giebultowicz. Integrative Biology, Oregon State University, Corvallis, OR.

Circadian clocks coordinate behavioral, neurological, and physiological processes into circa 24-hour rhythms. In *Drosophila melanogaster*, the central clock neurons directly control locomotor activity rhythms. However, several other cells such as photoreceptors and glia express clock genes in a rhythmic manner, yet the roles of these peripheral clocks are much less understood. It has been shown that aging is associated with reduced expression of clock genes in fly heads. In addition, loss of clock function in all tissues such as in *period*-null mutants show accelerated aging phenotypes. Together, these data suggest that strong circadian clocks may delay aging in flies. However, it is not known which clock expressing cells are important for delayed aging phenotypes. Glial cells are crucial for neuronal homeostasis by providing insulation and modification of the neuronal microenvironment, yet the roles of glial clocks are not understood. Using behavioral and molecular techniques, we investigate whether the clock gene expression in glia may be important in the aging process. To address this, we investigate whether RNAi mediated knockdown of clock genes such as *period*, *timeless*, and *cycle* in glia accelerates aging phenotypes. Preliminary activity data show that glia targeted clock gene RNAi decreases the percentage of rhythmic males compared to age-matched controls. Tests of climbing ability of glial *period* knockdown flies at different ages show accelerated functional decline in vertical climbing ability compared to age-matched controls. Our experiments may reveal previously unknown age-related circadian functions of glial cells.

547C

Sleep abnormalities in a *Drosophila* knock-in model of human generalized epilepsy with febrile seizures-plus (GEFS+). Emily Petruccelli, Patrick Lansdon, Toshihiro Kitamoto. University of Iowa, Iowa City, IA.

Despite an established link between epilepsy and sleep behavior, it remains unclear exactly how specific epileptogenic mutations affect sleep and how sleep influences epileptic seizures. *Drosophila* is an attractive model for studying the underlying mechanisms of this seizure/sleep relationship as it is routinely used to examine the genetic basis of seizure susceptibility and sleep behavior. Sun *et al* (2012) recently created a knock-in fly model of human generalized epilepsy with febrile seizures plus (GEFS+), a wide spectrum

disorder characterized by fever-associated seizing in childhood and lifelong affliction. GEFS+ flies carry a mutation in the voltage-gated sodium channel (Na_v) gene, mimicking a disease-causing human Na_v mutation (*SCN1A*^{K1270T}) and display a semidominant heat-induced seizure phenotype as a result of abnormal electrophysiology in inhibitory GABAergic neurons. We found that GEFS+ mutation also dominantly modifies sleep behavior, with mutants exhibiting rapid sleep onset at dusk and increased nighttime sleep as compared to controls. This sleep profile was observed regardless of sex, mating status, and genetic background. Mutants' exaggerated sleep was more resistant to carbamazepine (CBZ), a drug that reduces *Drosophila* $GABA_A$ receptor activity, and could be suppressed by either constant or acute scotophase light. We further observed that GEFS+ flies have normal circadian rhythm in free-running dark conditions, but significantly lack homeostatic rebound following sleep deprivation. Intriguingly, sleep deprivation treatment increased the heat-induced seizure susceptibility of control flies, but reduced the seizure severity of GEFS+ mutants. Ongoing experiments are addressing the potential significance of GABAergic inhibition on wake-promoting PDF+ neurons in GEFS+ mutant sleep and the impact of seizing on subsequent sleep behavior. Our findings thus far have characterized the sleep architecture of *Drosophila* harboring a human GEFS+ mutation and provided unique insight into the relationship between sleep and epilepsy.

548A

Neurexin regulates sleep by modulating the Na^+/K^+ ATPase activity. Huawei Tong, Qian Li, Wei Xie, Junhai Han. Institute of Life Sciences, Southeast University, Nanjing, Jiangsu, China.

Sleep is a universal, essential and evolutionarily conserved behavior among animal species. Converging lines of evidence strongly suggested that sleep behavior is closely related to synaptic function. Neurexins are cell adhesion molecules and function in synapse formation and synaptic transmission. However, the potential role of Neurexins in sleep behavior is unknown. Here, we show that lack of the*Drosophila* homolog of α-Neurexin lead to significantly impaired sleep behavior. Depletion of Neurexin in mushroom body recaptured the defective sleep behavior observed in neurexin mutant flies, and expression of Neurexin in mushroom body successfully restored the impaired sleep in *neurexin* mutants. Furthermore, we revealed that the defective sleep behavior in *neurexin* mutant flies is due to the altered synaptic neurotransmission. Using yeast two-hybrid screening, we identify that Neurexin interacts with Nrv3, the β subunit of Na^+/K^+ ATPase. Finally, we demonstrated that Neurexin regulate sleep behavior in mushroom body through modulating the function of Na^+/K^+ ATPase. Our study reveals an unknown mechanism of Neurexin in modulation synaptic transmission and in regulating sleep behavior, and offers an insight into the mechanism of sleep disturbances in autism patients.

549B

Circadian Translational Profiling Of The Drosophila Head Fat Body. Amy Marie Yu, Yanmei Huang, F. Rob Jackson. Neurobiology, Tufts University Medical School, Boston, MA.

Flies exhibit rhythmicity in many behaviors, including locomotion, feeding, and courtship, as well as in phenotypes such as toxin and infection susceptibility. The transcription-translation based feedback oscillator that directs these rhythms is well understood, but the pathways wherein the clock signal is transformed into phenotypic rhythms have not been as clearly defined. Analysis of clock output by methods such as microarray or RNA-Seq is complicated by the fact that many different cell types harbor autonomous molecular clocks. In the Drosophila head, for example, clock cells comprise neurons, glia, photoreceptors, and fat body cells. Presumably, these clocks all serve cell type specific functions; however, most previous studies of rhythmic RNA abundance in flies have been done in extracts from bulk tissue. Thus, the cell type specific roles of fly molecular clocks remain poorly understood. To investigate the cell type specific roles of biological clocks in Drosophila, we have used translating ribosome affinity purification (TRAP) to selectively isolate mRNAs from target cell populations for circadian analysis. In TRAP, a GFP-tagged ribosomal subunit is selectively expressed in the cell type of interest, allowing immunoprecipitation of associated mRNA. Recently published work from our lab has shown that TRAP enhances sensitivity for detecting cycling mRNAs in bulk tissue for genes expressed at high levels in both clock and non-clock cells. We are now using fat body specific Gal4 drivers to examine the molecular clock and rhythmic mRNAs in the Drosophila head fat body. Using TRAP and bioinformatic analysis, we have identified a set of approximately 200 genes expressing mRNAs which are both enriched and cycle in the head fat body. Many of these genes cluster in functional groups related to immunity, wound healing, energy metabolism, and sex-specific physiology. Several of these fat body enriched cycling mRNAs have not been previously shown to cycle in abundance. Studies are currently underway to determine whether the cycling of these mRNAs depends solely on the fat body clock or, alternatively, if there is a requirement for output from other clocks of the Drosophila head.

550C

Genetic dissection of aggressive behavior in *Drosophila melanogaster*. Mahmoudreza Ramin, Claudiu Domocos, David Slawaska-Eng, Yong Rao. Centre for Research in Neuroscience, Department of Neurology and Neurosurgery, McGill University Health Centre, Montreal, Quebec, Canada.

Aggression is an innate behavior that helps animals survive. It occurs when animals compete for limited resources, such as food, mating partners and habitats. Accumulated evidence supports that the control of aggressiveness involves both genetic and epigenetic factors. Recent studies have begun to reveal molecular and cellular mechanisms that modulate the level of aggressiveness in experimental model systems such as Drosophila and mice. We utilize Drosophila as a model to understand the mechanisms underlying the control of aggressive behavior. To investigate if visual perception is required for social suppression of fly aggression, we manipulated visual circuit activity and examined its effects on the behaviors of grouped flies. Our results showed that the blockade of visual circuit activity does not affect social suppression of aggression. We also found that temporal blockade of vision significantly increased the level of aggressiveness of flies without social experience.

Moreover, to gain insights into molecular networks that control fly aggression, we performed a systematic genetic screen to identify genomic regions that are involved in the suppression of fly aggression. This suggested us some potential candidates for further studies.

551A

Molecular and behavioral analysis of gustatory receptor neurons in pharyngeal organs as modulators of feeding in *Drosophila* larvae. Jaekyun Choi, Min Sung Choi, Jae Young Kwon. Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, South Korea.

Finding and feeding on food sources is necessary for the survival of all animals. This behavior is mainly influenced by the taste and nutrition of a food source, as well as the nutritional status of an individual organism. Sweet substances generally cause attractive behavior, and bitter substances generally cause aversive behavior. Feeding behavior can be defined as the entire process that encompasses searching for food to digestion and nutrient absorption. We are using the *Drosophila* larvae as a model system to study the process of choice, in which the quality of food is judged, and the process of ingestion, in which the selected food is ingested into the digestive tract. We tested 23 bitter compounds for the larvae response in choice and ingestion as separate processes. Several compounds showed different behavioral responses in terms of choice and ingestion. For example, although denatonium causes an aversive response in a choice assay, it has a neutral effect on ingestion. To examine the functions of gustatory receptors and gustatory receptor neurons in *Drosophila* larvae, we used a molecular genetic approach as well as behavioral assays. From our analyses, it appears that the terminal organ, the major taste organ of *Drosophila* larvae, mainly acts in recognizing aversive chemicals and eliciting avoidance behavior, while the pharyngeal organs appear to regulate both avoidance behavior based on choice as well as ingestion. In addition, we have defined in detail the differences in brain projections of pharyngeal gustatory neurons that positively enhance ingestion and pharyngeal gustatory neurons that negatively suppress ingestion. This work was supported by the National Research Foundation of Korea Grant NRF-2011-0017239.

552B

The regulation of feeding and metabolism by activating insulin signaling in DSK neurons in *Drosophila*. Justin Palermo, Justin R. DiAngelo. Department of Biology, Hofstra University, Hempstead, NY.

All organisms need to sense their nutritional environment and adjust their behavior accordingly. In *Drosophila*, several hormones and neuropeptides including the insulin-like peptides (dilps), the glucagon-like molecule adipokinetic hormone (AKH) and drosulfakinin (DSK), a cholecystokinin (CCK)-homolog, have been implicated in feeding, metabolism and altered activity in response to changes in food availability. For example, both DSK and dilp2 have been shown to act as satiety signals and the pathway that is activated in response to the dilps is well known for regulating nutrient storage and metabolism. However, whether DSK and insulin interact to control nutrient sensing, feeding and metabolism is not known. In this study, we activated insulin signaling by expressing a constitutively active form of the insulin receptor (dInRCA) in DSK-producing neurons and measured feeding and macromolecule storage. Expressing dInRCA in the DSK-producing neurons led to an increase in feeding as well as an increase in triglyceride levels. Interestingly, DSK mRNA was unchanged when the insulin pathway was activated in DSK-producing neurons indicating that insulin signaling was not leading to altered DSK levels to result in the increased food consumption phenotype. However, the expression of *dilp2*, *3* and *5* was increased in flies with activated insulin signaling in the DSK neurons. This suggests that either the DSK neurons are communicating with the insulin-producing cells (IPCs) in the brain to regulate *dilp* levels or activating insulin signaling is leading to increased *dilp* expression in the same cells as several neurons in the brain produce both DSK and dilps. To differentiate between these possibilities, we expressed dInRCA specifically in the IPCs and observed no changes in *dilp* expression under these conditions suggesting that activating the insulin pathway in the subset of neurons that produce DSK, but not the dilps is important to regulate overall *dilp* expression in the fly brain. Together, these data support a role for insulin signaling in the DSK-producing neurons for regulating feeding and triglyceride storage as well as communicating with the IPCs to control *dilp* expression.

553C

The regulation of feeding and metabolism by the DSK receptor CCKLR-17D1 in *Drosophila*. Christopher Tenorio, Justin DiAngelo. Department of Biology, Hofstra University, Hempstead, NY.

The ability of an organism to recognize and store available nutrients as fat and glycogen is essential for assuring its survival. Specific populations of neurons in the brain sense changes in nutrient availability leading to alterations in an animal's feeding behavior, amount of food consumed and energy expenditure. One neuropeptide that has recently been implicated in regulating feeding behavior in *Drosophila* is drosulfakinin (DSK). DSK inhibits feeding and is expressed in many different neurons including a subset of neurons in the pars intercerebralis (PI) that produces the *Drosophila* insulin-like peptides (dilps). However, the receptor that DSK acts on to control food consumption is unknown. In this study, we determined the effects of the putative DSK receptor CCKLR-17D1 on feeding and nutrient storage. *CCKLR-17D1* mutant females showed a decrease in feeding suggesting that this receptor promotes food consumption. Surprisingly, both glycogen and triglyceride levels are increased in the *CCKLR-17D1*mutants, suggesting that the decreased feeding phenotype in these flies may be compensatory to changes in nutrient storage. The feeding phenotype of the *CCKLR-17D1* mutants was also likely not due to an increase in DSK levels as quantification of DSK gene expression showed a decrease in *dsk* mRNA levels. Previous studies have shown decreases in *dsk* result in increased *dilp-2*, *dilp-3* and *dilp-5*mRNA. To determine whether *dilp* expression was altered in the *CCKLR-17D1* mutants, quantitative PCR for *dilp-2, 3* and *5* was performed on RNA from heads of *CCKLR-17D1* mutants. Interestingly,*CCKLR-17D1* mutants had higher *dilp-3* and lower *dilp-2* and *5* levels, potentially contributing to the feeding and nutrient storage phenotypes observed in the *CCKLR-17D1* mutants. Together, these data identify a role

for CCKLR-17D1 in regulating feeding and metabolism. In addition, since the phenotypes of the *CCKLR-17D1* mutant flies are not identical to flies where *dsk* is decreased, CCKLR-17D1 may act as a receptor for other peptides besides DSK. .

554A

Impaired climbing and flight behavior in *D. melanogaster* following carbon dioxide anesthesia. Nathan R Bartholmew[1], Jacob Burdett[1], John M VandenBrooks[2], Michael Quinlan[2], Gerald B Call[3]. 1) Arizona College of Osteopathic Medicine (AZCOM), Midwestern University, Glendale, AZ; 2) Dept of Physiology, AZCOM, Midwestern University, Glendale, AZ; 3) Dept of Pharmacology, AZCOM, Midwestern University, Glendale, AZ.

Laboratories that research *Drosophila melanogaster* use carbon dioxide (CO_2) on a daily basis to anesthetize flies for sorting and other work. However, CO_2has potential effects on a variety of physiological and behavioral mechanisms including respiratory and muscle physiology, and climbing and flight behavior. We have examined the effect of multiple levels of $CO_2$and varying exposure times on the subsequent recovery of motor function tested with both climbing and flight assays. We have found that with as little as five minutes exposure to 100% CO_2 *D. melanogaster* exhibit motor deficits up to 16 hours after exposure. Any exposure length above five minutes produces behavioral effects on climbing that last for over 24 hours. Overall, there is a positive correlation between carbon dioxide exposure length and recovery time. When given just one hour to recover after any length of exposure, climbing is reduced by 70-90% and flight by 40-60% as compared to control flies. This effect is not due to just anoxia, but a CO_2-specific mechanism as shown by a reduced impact of exposing *D. melanogaster* to just anoxia (100% N_2). Additionally, we have shown that exposure to as low as 65% CO_2(balanced with 20% oxygen and 15% nitrogen) affects the motor capability of *D. melanogaster*. Exposure of *D. melanogaster* to flow rates similar to that would be used in CO_2-exposure pads in most laboratories reduces climbing ability by 35-75% depending on the flow rate. These results point to a strong impact of CO_2 anesthesia on subsequent experimental test in the lab indicating the importance of monitoring CO_2 exposure levels, flow and length of exposure for any physiological or behavioral study.

555B

Anxiolytic-like effects of an organic extract from the seaweed *Sargassum platycarpum* in anxiety-related behavior in *Drosophila melanogaster*. Zulmari Martínez, Jesica Vicente, Angeliz Rivas, Keysh Mejías, Jeslie Ramos, Grisel Robles, Valeria Pedraza, Claudia Ospina, Ricardo Chiesa. University of Puerto Rico at Cayey, NEC Building Cayey, PR 00736.

Algae are aquatic organisms that present a great potential for the study of bioactive compounds. Several bioactive compounds with diverse effects have been found in species of algae from the three main groups (classes Phaeophyta, Rhodophyta, Chlorophyta). We hypothesize that whole organic extracts obtained from brown algae might possess compounds with the potential to decrease anxious behavior. An organic extraction was made using the seaweed *Sargassum platycarpum,* collected from a beach in the south shore of Puerto Rico. The anxiolytic-like effects of the whole extract was tested using*Drosophila Melanogaster* as the anxiogenic model. Anxiety-like behavioral traits in *Drosophila* were achieved using the Open Field Arena (OFA) paradigm (phobic behavior), and by the development of insomnia by food deprivation. Before exposure to the algal extracts, both anxiety-like behaviors were validated to make certain that the flies presented the expected behavioral characteristics. The whole organic extract from *Sargassum platycarpum* was dissolved in 23 % Dimethyl Sulfoxide (DMSO) and toxicity tests both for DMSO and the whole organic extract were performed before the behavioral tests. For the behavioral tests, 1 ml of whole extract was added to 5 ml of food, and adult flies exposed to food with the extract since larval and pupal stages were compared to control groups (flies not exposed to the extract). Differences in behavior were assessed by video recordings and quantitative measurements. Flies exposed to the whole algal extract show a decrease in the phobic/aversive behavior characteristic of the OFA test and also decreases insomnia in food deprived flies. In conclusion, though we have to further validate these preliminary results, *Sargassum platycarpum* whole organic extract exerts anxiolytic-like effects in a *Drosophila melanogaste*r model of anxiety.

556C

Ethanol-Induced locomotion in *Drosophila melanogaster* is decreased by the administration of an organic extract of the seaweed *Sargassum platycarpum*. Adbiel Rodríguez, Noelia Acevedo, Karelis Pagán, Claudia Ospina, Ricardo Chiesa. University of Puerto Rico at Cayey Antonio R. Barcelo Avenue NEC Building Cayey, PR 00736.

There is a lower incidence and prevalence of anxiety and other behavioral disorders in places where algae is consumed regularly. The study and characterization of bioactive compounds in algae has been of great interest for scientists in the last two decades. We have selected *Drosophila melanogaster* as a model to study the effects of alkaloid extracts of brown algae of Puerto Rican coasts in anxiety related behaviors. This model offers several advantages for behavioral and pharmacological studies, not only because of its size, short life span, and easy maintenance, but also because of its similarities in cellular and neurobiological processes with vertebrate animals. In order to study drug-induced anxiety-related behavior in flies we have administered ethanol, which increases locomotion in *Drosophila Melanogaster*. This behavior serves as a paradigm to measure the anxiolytic-like effects of the brown algae's extract used in this project. After administering ethanol to observe the flies' behavior, we can conclude we have validated our paradigm of anxiety-related behavior induced with ethanol. To study the possible anxiolytic-like effects in flies exposed to ethanol, *Drosophila melanogaster* was exposed to a whole organic extract of *Sargassum platycarpum* over a two week period. After two weeks of exposure of *Drosophila Melanogaster* to food containing the organic extract of *Sargassum Platycarpum*, the effects of its behavior in locomotion was evaluated by video recordings and quantitative analysis. Preliminary data shows a decrease in locomotion in *Drosophila Melanogaster* that was given the extract before ethanol exposure. Further analysis is required to validate this behavior. If anxiolytic effects are found in the brown algae's extract, this project opens future possibilities to create a new pharmacological treatment for anxiety-related behavior.

557A

New genes required for mechanical nociception identified by a novel assay for mechanical pain sensitization. PJ Huang[1,2], HT Turner[2], SH Im[2], MJ Galko[2]. 1) Department of Biochemistry & Cell Biology, Rice University, Houston, TX; 2) Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX.

Pain sensitization is a local change in pain threshold induced by tissue injury. It can occur as allodynia, the perception of an innocuous stimulus as noxious, or hyperalgesia, an exaggerated response to a noxious stimulus. To better understand the mechanisms of mechanical pain sensitization, we studied it using *Drosophila* larvae. *Drosophila* larvae exhibit an aversive 360º rolling response along their body axis when poked with high-force "harsh-touch" noxious probes. We modified previous assays for baseline mechanical nociception by constructing a graded series of customized larval Von Frey filaments of different diameters and lengths that exert constant pressures upon bending. We showed that mechanically induced aversive rolling behavior was first observed in a minority of larvae with a 300 kPa probe; pressures below this threshold did not induce aversive rolling. Probes spanning 300 kPa to 4900 kPa resulted in increasingly penetrant rolling behavior until nearly all larvae responded. By comparing behavioral responses from paired filaments of identical pressures with filaments of identical forces, we found that pressure was the primary determinant of the observed behavioral response. To identify genes required for mechanical nociception, transient receptor potential (TRP) channel mutants were tested for their roles in baseline mechanical sensation. *TRPM, brivido1, painless, and piezo* mutants were hyposensitive to noxious mechanical stimuli. Furthermore, UV-induced epidermal tissue damage triggered both mechanical hyperalgesia and allodynia. This sensitization following tissue damage was abolished by electrically silencing class IV sensory neurons with a tetanus toxin transgene, which blocks synaptic transmission, indicating that UV-induced sensitization to mechanical pain occurred in class IV sensory neurons. Moreover, mechanical sensitization was independent of three pathways (TNF, Hedgehog, Tachykinin) known to cause thermal hypersensitivity in both *Drosophila* and vertebrates. These studies establish a valuable new assay for genetic dissection of mechanical pain sensitization.

558B

Sensory control of larval epithelial cell layer redox status through ROS-mediated avoidance of atmospheric oxygen to maintain foraging stage food immersion. Wayne Johnson, Carder Justin. Dept Molec Physiol/Biophysics, Univ Iowa, Iowa City, IA.

Sensory neurons innervating vertebrate respiratory epithelial cell layers respond to atmospheric oxygen-derived oxidants as well as a variety of chemical and environmental insults to mediate a protective reflex for sneezing, cough and pain. However, excessive levels of reactive-oxygen species(ROS) in respiratory epithelia can contribute to common respiratory pathologies such as asthma and chronic obstructive pulmonary disease. *Drosophila* larval class IV multiple dendritic(mdIV) sensory neurons innervating epithelial cell layers of the larval body wall serve as a useful genetic model for understanding the molecular and physiological mechanisms for sensory neuron responses to ROS. Previous work has shown that the mdIV neurons are activated by nanomolar levels of H_2O_2 consistent with a highly sensitive role in monitoring redox status in the larval epithelia analogous to the sensory monitoring of vertebrate respiratory epithelia. Results presented here show that the mdIV neurons function to maintain food immersion of foraging stage larvae through an ROS-mediated oxygen aversion behavior. Transient inactivation of mdIV neurons in ppk1-GAL4/UAS-shi^{ts1} foraging stage larvae causes a rapid premature larval exit from the food. Transgenic epithelia-specific overexpression of catalase to breakdown endogenous H_2O_2 causes the same premature food exit that is suppressed by increasing atmospheric oxygen levels. Catalase-stimulated premature food exit is significantly enhanced in a background with decreased dosage of the DEG/ENaC subunit Pickpocket1(PPK1) suggesting a mechanistic link. Transgenic disruption of PPK1 function in mdIV neurons of foraging stage larvae using the dominant-negative isoform, PPK1(E145X) or a UAS-ppk1RNAi caused a strong premature food exit consistent with a key role for PPK1. Knockdown of endogenous TrpA1 expression using TrpA1-RNAi did not cause premature food exit. These initial results are consistent with a model in which H_2O_2 produced from atmospheric oxygen in the epithelia and/or adjacent cuticle acts as a signal to activate mdIV neurons mediating the oxygen aversion behavior resulting in maintenance of complete food immersion during foraging stages. .

559C

Factors affecting the release of and response to the Stress Odourant (dSO) by *Drosophila melanogaster*. Ian S McDonald, Selwyn Chui, Andrew F. Greco, Shirley Q. Long, Jeremy N. McNeil,**Anne F. Simon**. Department of Biology, Faculty of Science, Western Ontario University, Ontario, London, Canada.

Plants and animals obtain vital information that influences a number of behaviours, including resource acquisition, reproduction, and predator evasion, through auditory, visual, tactile, and chemical cues. Insects commonly utilize chemical signals that cause avoidance responses in conspecifics. For example, alarm pheromones warn conspecifics of potential danger and induce behavioural changes that reduce the probability of injury or death. *Drosophila melanogaster* adults avoid areas previously occupied by stressed conspecifics in response to a '*Drosophila* Stress Odour' (dSO). Here, we are interested in determining the ecological relevance of dSO. Our data suggest that it may serve as an alarm cue, as dSO is emitted by flies regardless of age, sex, and mating status. In addition, a detectable amount of dSO is also released by just one fly. Finally, flies emit dSO merely when transferred into a vial, but not when moved through phototaxis, which is relevant information for any researcher performing behavioural analysis. We also are interested in how dSO affects responders, and will present data on how flies respond to food odour in the presence of dSO. By characterizing the conditions underlying the emission and reception of dSO, we will be able to pursue several directions. We will use this assay as a diagnostic tool to assess a form of social behaviour: the flies' response to others being stressed. We will also be able to identify the chemical component(s) of dSO (beside CO_2), and we will dissect the neural circuitry underlying this behaviour.

560A

Reduced odor evoked neural responses during sleep and impaired response after prolonged waking in the Drosophila mushroom body (MB). Daniel B Bushey, Giulio Tononi, Chiara Cirelli. Psychiatry, University of Wisconsin, Madison, WI.

Local field potential recordings found reduced activity in the *Drosophila* brain during sleep compared to wake, but activity and responses to stimulation at the individual neuron level have not been studied. Using GCaMP5, we monitored Ca levels in individual MB cells while monitoring leg and abdomen activity to assess whether the fly was asleep or awake. First, we assessed Ca levels in single flies kept in constant environmental conditions over 2h periods. Following standard behavioral scoring, a fly was considered asleep if no leg/abdomen activity occurred during imaging and in the 2min before and after imaging. To promote sleep, recording occurred at night when flies normally sleep. As expected, flies slept more after being sleep deprived before testing with increased sleep episode duration and number. Further, the infrared laser had an arousing effect, with flies moving 59% of the time when the laser went on, but only 36.3% of the time during mock periods with no imaging. Crucially, flies already active before imaging moved 81.3% of the time (n=101 sessions) when the laser went on, while if quiescent before testing they only moved 39.2% of the time, confirming that sleep as behaviorally defined here is associated with increased arousal thresholds. During baseline spontaneous activity is low in the mushroom body but some cells still fluoresced brightly. Ca levels dropped (0.16 ΔF/F) in these cells in transitions from wake to sleep and increased (1.7 ΔF/F) in transitions from sleep to wake. We then compared evoked responses to oxygen enriched and vinegar odor streams tested over a 20min period. Here, comparisons were made between flies that remained awake or asleep during testing. Fewer cells responded to odors during sleep as compared to wake, and their Ca response was smaller. Lastly, we also tested whether the odor specific activation pattern in awake animals changes after sleep (5-8h), short term wake (5-8h) or prolonged waking (29-34h). Pearson's correlation found a consistent activation pattern after sleep and short term wake, while after prolonged waking the pattern became inconsistent, with fewer cells responding with high intensity Ca transients after prolonged wake compared to after sleep. .

561B

The Effect of Bacteria on Oviosition Preference of *Drosophila melanogaster*. Geon Ho Kim[1], Peter Newell[2], Angela Douglas[1]. 1) Entomology, Cornell University, Ithaca, NY; 2) Microbiology, State University of New York, Oswego, NY.

For insects, finding a suitable egg-laying site is crucial for the fitness of their offspring. One of the most important aspects of the site should be its closeness to appropriate food source. Although there have been many studies on their egg-laying behavior, there has not been much study on the effects of bacteria and their metabolites on flies' oviposition preference. Therefore, we studied which types of food *Drosophila* prefers as an oviposition site, and tried to identify the cause behind such preference. As *Acetobbacters,* one of the main gut microbiota of *Drosophila melanogaster,* produces acetic acids as byproducts, and since flies preferred laying eggs on both acetobacters and acetic acids, we hypothesized that acids serve as a cue for the beneficial microbes. We showed organic acids production by bacteria through high performance liquid chromatography (HPLC). In addition, as gnotobiotic flies are reported to develop at faster rate than axenic flies, we hypothesized that rearing axenic flies in the food mixed with organic acids would also expedite larval development. Interestingly, results showed that pupation was delayed significantly when axenic flies were reaised in the acids. As delayed development can significantly compromise fitness of the flies, such results suggests that organic acids may be cues for beneficial microbes, but brings detrimental effects when consumed without those bacteria. .

562C

Complex and non-redundant signals from individual odor receptors that underlie chemotaxis behavior in *Drosophila melanogaster* larvae. Jeewanjot Grewal, Christina Cho, Karolina Kir, Nicole Fledderman, Kathryn Swain, **Scott A. Kreher**. Department of Biological Sciences, Dominican University, River Forest, IL.

The rules by which odor receptors encode odors and allow behavior are still largely unexplored. Although large data sets of electrophysiological responses of receptors to odors have been generated, few hypotheses have been tested with behavioral assays. We use a data set on odor responses of Drosophila larval odor receptors coupled with chemotaxis behavioral assays to examine rules of odor coding. Using mutants of odor receptors, we have found that odor receptors with similar electrophysiological responses to odors across concentrations play non-redundant roles in odor coding at specific odor concentrations. We have also found that high affinity receptors for odors determine behavioral response thresholds, but the rules for determining peak behavioral responses are more complex. While receptor mutants typically show loss of attraction to odors, some receptor mutants result in increased attraction at specific odor concentrations. The odor receptor mutants were rescued using transgenic expression of odor receptors, validating assignment of phenotypes to the alleles. Vapor pressures alone cannot fully explain behavior in our assay. Finally, some odors that did not elicit strong electrophysiological responses are associated with behavioral phenotypes upon examination of odor receptor mutants. This result is consistent with the role of sensory neurons in lateral inhibition via local interneurons in the antennal lobe. Taken together, our results suggest a complexity of odor coding rules even in a simple olfactory sensory system.

563A

Presynaptic gain control drives sweet and bitter taste integration. Bonnie Chu, Vincent Chui, Kevin Mann, **Michael Gordon**. Zoology, University of British Columbia, Vancouver, British Columbia, Canada.

The sense of taste is critical in determining the nutritional suitability of foods. Sweet and bitter are primary taste modalities in mammals and their behavioral relevance is similar in flies. Sweet taste drives the appetitive response to energy sources, while bitter taste drives avoidance of potential toxins and also suppresses the sweet response. Despite their importance to survival, little is known

about the neural circuit mechanisms underlying integration of sweet and bitter taste. Here, we describe a presynaptic gain control mechanism in *Drosophila* that differentially affects sweet and bitter taste channels and mediates integration of these opposing stimuli. Gain control is known to play an important role in fly olfaction, where $GABA_B$ receptor ($GABA_BR$) mediates intra- and interglomerular presynaptic inhibition of sensory neuron output. In the taste system, we find that gustatory receptor neurons (GRNs) responding to sweet compounds express $GABA_BR$, while those that respond to bitter do not. $GABA_BR$ mediates presynaptic inhibition of calcium responses in sweet GRNs, and both sweet and bitter stimuli evoke GABAergic neuron activity in the vicinity of GRN axon terminals. Pharmacological blockade and genetic reduction of $GABA_BR$ both lead to increased sugar responses and decreased suppression of the sweet response by bitter compounds. We propose a model in which GABA acts via $GABA_BR$ to expand the dynamic range of sweet GRNs through presynaptic gain control, and suppress the output of sweet GRNs in the presence of opposing bitter stimuli.

564B

Identification and characterization of gustatory second-order interneurons. K. Shimizu[1], T. Miyazaki[1], T.Y. Lin[1], K. Ito[2], C.H. Lee[1], M. Stopfer[1]. 1) NICHD/NIH, Bethesda, MD 20892, USA; 2) IMCB, Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032, Japan.

An important function of the nervous system is to transform the format of information as it moves through layers of neural populations so that the information can be effectively used by the brain. For an animal to survive, its gustatory system, much like its olfactory system, has to detect and encode information about chemicals present in the environment. A prominent feature of these chemosensory systems is their ability to encode the extremely high-dimensional information needed to characterize these chemicals. How is such high-dimensional information encoded and interpreted by the brain? Insect chemosensory systems serve as good models to answer this question as they are simpler than yet substantially similar to that of mammalian counterparts. In the *Drosophila* gustatory system, soluble chemicals are detected by the gustatory sensory neurons (GSNs) distributed on various body appendages, including the mouthparts, legs and wings. The neural encoding in this first layer of the gustatory system has been characterized anatomically and physiologically in many ways by leveraging the powerful genetic tools available to study *Drosophila*'s nervous system. However, the neural basis of gustatory information processing downstream from the peripheral layer remains largely unknown. As the first step to elucidate neural encoding in the fly gustatory system, we searched for gustatory interneurons receiving direct synaptic input from GSNs (i.e., 2^{nd} order neurons). First, we performed a Gal4 screen for interneurons projecting to the subesophageal ganglion (SEG), where the GSNs send their axons, followed by a further screen with the GRASP method to check for synaptic connections between the GSNs and the labeled interneurons. We next used Ca^{2+} imaging techniques to characterize the responses of anatomically identified gustatory 2^{nd} order interneurons to tastants. So far, we have identified a single class of gustatory 2^{nd} order neuron that responds to multiple tastants. We will discuss possible coding mechanisms and the reformatting of gustatory information in the 2^{nd} layer of gustatory neural population.

565C

Ecdysis Triggering Hormone Receptor Splice Isoforms Govern Distinct Aspects of *Drosophila* Ecdysis Sequences. Feici Diao[1], Fengqiu Diao[1], Wilson Mena[2], Brandon Mark[2], Jonathan Shi[3], Dongkook Park[3], Paul Taghert[3], John Ewer[2], Benjamin White[1]. 1) national institute of mental health, Bethesda, MD; 2) CINV, Universidad Valparaiso, Valparaiso, Chile; 3) Washington University, St Louis.

Ecdysis Triggering Hormone (ETH), which activates the ecdysis motor sequences that cause the shedding of the exoskeleton at each insect molt, provides a striking example of how hormones can profoundly shape behavior by inducing activity in widely distributed neural networks. Among the networks are diverse ensembles of identified peptidergic neurons that express the A isoform of the ETH receptor (ETHRA). However, the activity in these networks accounts for only some ecdysis behaviors and only at some molts. Other targets of ETH include ETHRA-expressing neurons outside the known ensembles as well as neurons that express a second ETHR splice isoform, ETHRB. The function of these targets in ecdysis has remained unknown. Using a novel technique to generate Gal4 drivers that express in all ETHR-expressing neurons, or selectively in ETHRA- or ETHRB-expressing neurons, we find that the two ETHR isoforms and the neurons that express them play distinct roles in ecdysis at both the larval and pupal stages. Mutation of the ETHR gene results in lethality at larval ecdysis that is rescued by expression of a UAS-ETHRA, but not a UAS-ETHRB, transgene under the control of ETHR-Gal4. Suppression of activity in ETHRA expressing neurons causes 100% lethality at larval ecdysis. ETHRB and the neurons that express it are, in contrast, dispensable for larval ecdysis. Mutagenesis of ETHRB, or suppression of activity in ETHRB-expressing neurons causes failures at the ecdysis to pupal stage. This failure results from behavioral arrest prior to head eversion (pre-ecdysis). We find that at the pupal stage ETHRA-expressing neurons have no essential function in pre-ecdysis behaviors, but are required for head eversion. Overall, our results demonstrate that the motor programs governing larval and pupal ecdysis differ in their requirements for the two ETHR isoforms and the neurons that express them, and that ETHRA- and ETHRB-expressing neurons govern distinct aspects of the pupal ecdysis sequence. .

566A

Virtual Fly Brain - A Data Integration Hub for *Drosophila*Neurobiology. David J. Osumi-Sutherland[1], Marta Costa[2], Robert Court[3], Gregory S.X.E. Jefferis[4], Helen Parkinson[1], J. Douglas Armstrong[3], Cahir J. O'Kane[2]. 1) EMBL-EBI, Cambridge, UK; 2) Dept of Genetics, University of Cambridge, Cambridge, UK; 3) School of Informatics, University of Edinburgh, Edinburgh, UK; 4) MRC Laboratory for Molecular Biology, Cambridge, UK.

Informatics support is essential to successful mapping and genetic dissection of the *Drosophila* nervous system. Virtual Fly Brain (VFB) is an open, online resource that plays a central role in providing this informatics support. Through our website (www.virtualflybrain.org), users can search and query for referenced descriptions of neuron classes, lineage clones, expression patterns and phenotypes, curated

from tens of thousands of publications. This information is integrated with FlyBase and thus with wider genetic, genomic and phenotypic information. VFB features over 20,000 3D images of neurons, expression patterns and neuronal clones from different published sources. These are registered to a standard brain, with neuropils and tracts painted according to the BrainName standard. This allows users to combine and compare images from multiple sources within the VFB image browser and to assess their relationship to standard anatomy. VFB also incorporates the results of image analysis, including clustering of morphologically similar neurons based on a unique morphology similarity scoring system (NBLAST) developed by GJ. Clusters can be viewed using a rotatable 3D image browsing system. Many clusters correspond to known neuron classes and work to add these mappings to our database is ongoing. We are currently overhauling our website while massively increasing content. The new site will allow queries starting from images, genes, transgenes and phenotypes, as well as from anatomical structures and neurons. Queries from images will include predictions of which neurons are contained within expression patterns and vice- versa. The site will incorporate an interactive 3D viewer and a social layer allowing users to comment and vote on the accuracy of all content.

567B

Failure to burrow while wandering: an indication of tracheal damage in mutants of the *jim lovell* (*lov*) gene. K. Beckingham, F. Zhou, K. Qiang, Y. Yuan, P. Yun, R. Dibbs, T. Ghanayem, R. Mohan, P. Frasse, N. Sankoorikkal. BioSciences, Rice University, Houston, TX.

At the end of larval life, Drosophila larvae leave their food and wander for a day or so before pupating. We examined this phase of the life cycle using larvae reared in yeast mounds at the center of 2% agar plates. Wandering wild type larvae prefer to burrow through the agar rather than wander on its surface. This behavior produces striking 'burrowing tracks" in the plates. In investigating selective RNAi knockdown of the gene *jim lovell* (*lov*) we discovered that certain Gal4-*lov* RNAi combinations produced larvae that i) would not enter the yeast mounds to feed and ii) stayed on the agar surface rather than burrowing into it during the wandering phase. Flushing the plates with nitrogen caused wandering larvae to come out of their burrows, indicating that the lack of burrowing is a response to hypoxia. Similarly the failure to enter the food is highly reminiscent of the response to hypoxia described previously by the O'Farrell lab (Wingrove & O'Farrell Cell 98 p.105, 1999). Most of the Gal4-*lov* RNAi combinations that produce these effects also produce defective tracheae that are partially or completely filled with liquid. *lov* encodes a BTB-POZ domain transcription factor and our studies indicate that it regulates expression of some structural proteins of the tracheae. However a *lov*-Gal4 line that expresses in both the tracheae and certain sensory neurons produces fluid-filled tracheae without the hypoxia-like behavior. This finding suggests an additional role for *lov* in oxygen sensing. Further studies with other mutations that damage the tracheae and with mutations affecting oxygen sensing are in progress.

568C

Differential regulation of *Drosophila* Glutamate receptor subunit production by *optimus-prime*, a novel mRNA associated gene. Dina M. Beeler[1], Julie E. Karr (Minibiole)[2], Subhashree Ganesan[1], David E. Featherstone[1]. 1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Department of Science and Mathematics Columbia College Chicago, Chicago, IL.

Postsynaptic receptor abundance is a critical determinant of synapse strength. We are identifying and studying mechanisms that control glutamate receptor (GluR) abundance in *Drosophila* embryonic and larval neuromuscular junctions (NMJ). Regulation of the production, trafficking, stability, and translation of GluR mRNA appears to be of particular importance in controlling GluR abundance. We have shown GluR subunit mRNA in embryonic and larval NMJs is associated with messenger ribonucleoprotein (mRNP) complexes, which are distributed throughout the cytoplasm of postsynaptic muscle cells. A novel protein was identified in a biochemical screen that appears to associate specifically with *GluRIIA* mRNA and regulate GluRIIA protein abundance. We named this novel gene, CG12149, '*optimus-prime* (*opr*)'. Mutants and muscle-specific RNAi of *OPr* leads to loss of GluRIIA protein but no change in *GluRIIA* mRNA quantity or loss of other GluR subunits. A polyclonal antibody raised against OPr shows immunoreactivity distributed throughout the central nervous system and muscle cells. Optimus prime is shown to be highly conserved and is the founding member of a novel protein family. SNPs in the human opr homolog are associated with autism spectrum disorders. .

569A

Early life stress affects higher order procesisng in brain structures in *Drosophila*. Davinelle Daniels[1], Andres Nieto[2], Wendi Neckameyer[2]. 1) Academic Affairs, Harris-Stowe State University, St. Louis, MO; 2) Dept of Pharmacological & Physiological Science, Saint Louis University School of Medicine.

Early life stress (ELS) occurs before the onset of sexual maturity and increases the risk for affective disorders in adults. It is believed that exposure to stress at vulnerable periods of synaptic organization modifies the development of response circuits, resulting in this enhanced risk. We have established a model for ELS in the fruit fly, *Drosophila melanogaster*. Newly eclosed (0-2 hour old) flies were exposed to four different stressors (starvation, oxidative stress, sleep deprivation and social isolation) for 24 hr and aged for 5 days. The animals were then analyzed for innate behaviors (measured in an open field arena, a light dark box, a Forced Swim Test and for the sedative responses to ethanol), cognitive abilities (habituation, egg-laying choice) and structural changes in the mushroom bodies and ellipsoid body. Innate behaviors are largely unaffected, and female egg-laying choice was also unaffected. However, changes in mushroom body and ellipsoid body size after exposure to ELS are sexually dimorphic, and males exposed to ELS fail to learn in the habituation paradigm. Thus, the fly brain is not completely "hard-wired," but, like mammals, is capable of significant structural plasticity in response to a changing environment.

570B

The Role of the BMP signaling family in the Induction of Allodynia. Taylor Follansbee[1], Kayla Gjelsvik[1], Michael Galko[2], Geoffrey Ganter[1]. 1) University of New England, Biddeford ME; 2) UT Southwestern MD Anderson, Houston TX.

The NIH has recently stated that in the United States alone over 100 million people are affected by chronic pain and unfortunately there is a lack in scientific understanding of the mechanisms of increased pain sensitivity. The present study utilized a candidate gene approach to identify novel components required for modulation of the pain sensitization pathway in *Drosophila melanogaster*. Our genes of interest are members of the Bone Morphogenetic Protein (BMP) signaling pathway. BMP is a member of the TGF family. Other members of the TGF family have been investigated for their role in sensitization, while the BMP family has remained overlooked. In this study, a protocol of UV-induced sensitization was performed on groups of 90 larvae compared to 90 "mock-sensitized" control larvae. We then systematically knocked down expression using the Gal4-UAS-RNAi system to target members of the BMP family in the primary nociceptor neurons. The knockdown of BMP ligands significantly attenuated the formation of sensitization compared with controls. These manipulations did not affect the normal nociceptive behavior of the fly, nor did it affect the morphology of the nociceptor neurons. These results indicate that the role of BMP in pain modulation is specific to the sensitization pathway. Up-regulation of BMP in the primary nociceptors genetically induced sensitization in the larvae compared with controls. This indicates that the BMP family is both necessary and sufficient for the formation of sensitization of *Drosophila melanogaster*. These results support future studies to determine which downstream receptors and what portions of the SMAD cascade are necessary for the development of sensitization. .

571C

Sex peptide receptor and myoinhibitory peptide modulate mating state-dependent choice behavior of *Drosophila* females. Ilona C Grunwald Kadow[1], Ashiq Hussain[1], Mo Zhang[1], Habibe Ucpunar[1], Thomas Svenson[2], Elsa Quillery[2], Rickard Ignell[2]. 1) Sensory Neurogenetics Research group, Max-Planck Institute of Neurobiology, Martinsried, Germany; 2) Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Division of Chemical Ecology, Sundsvägen 14, 23053 Alnarp, Sweden.

Reproductive state influences physiological needs and with it the choice behavior of females of many species including humans. How mating and gravidity impinges on choice behavior is not well understood. Using the fly *Drosophila* as a model, we identify a neural mechanism that modulates female choice behavior according to their mating state. First, we show that polyamines (PA) represent important indicators of organic decay that female flies detect through specific olfactory and gustatory ionotropic receptors (IRs) to evaluate fruit maturity. Mated females exhibit strong attraction to volatile PA, but they avoid laying their eggs directly into PA-rich substrates. These choice behaviors are strongly reduced in virgins and sex peptide receptor (SPR) mutant females. Using targeted RNAi knockdown, we show that SPR and its alternative ligands, the myoinhibitory peptides (MIP), are required in chemosensory neurons to regulate the female's sensitivity to PAs. Furthermore, we show that this behavior is also conserved in mosquitoes. Females of the dengue fever vector Aedes aegypti also use PAs as indicators for optimal egg laying sites. Together, our data suggest that neuropeptide-mediated modulation of chemosensation mediates mating state-dependent choice behavior. Given that PAs have beneficial effects on reproduction and embryonic development of all animals including humans, our results might indicate a conserved mechanism of reproductive state-dependent choice behavior. .

572A

***Beadex* function in the motor neurons is essential for female reproduction in *Drosophila melanogaster*. Subhash Kairamkonda**, Upendra Nongthomba. Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India.

Drosophila melanogaster has served as an excellent model system for understanding the neuronal circuits and molecular mechanisms regulating complex behaviors. The Drosophila female reproductive circuits, in particular, are well studied and can be used as a tool to understand the role of novel genes in neuronal function in general and female reproduction in particular. In the present study, the role of *Beadex*, a transcription co-activator, in Drosophila female reproduction was assessed by generation of mutant and knock down studies. Null allele of *Beadex* was generated by transposase induced excision of P-element present within an intron of *Beadex* gene. The mutant showed highly compromised reproductive abilities as evaluated by reduced fecundity and fertility, abnormal oviposition and more importantly, the failure of sperm release from storage organs. However, no defect was found in the overall ovariole development. Tissue specific, targeted knock down of *Beadex*indicated that its function in neurons is important for efficient female reproduction, since its neuronal knock down led to compromised female reproductive abilities, similar to *Beadex* null females. Further, different neuronal class specific knock down studies revealed that *Beadex* function is required in motor neurons for normal fecundity and fertility of females. Thus, the present study attributes a novel and essential role for *Beadex* in female reproduction through neurons.

573B

Response to stress in *Drosophila* is mediated by the hormonal milieu of the brain. Wendi Neckameyer, Andres Nieto. Dept Pharmac & Physiol Sci, St Louis Univ School Med, St Louis, MO.

All living organisms must maintain equilibrium in response to internal and external challenges within their environment. Changes in neural plasticity are critical components of the homeostatic response to stress, which have been strongly implicated in the onset of affective disorders. However, stress is differentially perceived depending on the type of stress and its context, as well as genetic background, age and sex; therefore, an individual's maintenance of neuronal homeostasis, by definition, must differ depending upon these variables. We have established *Drosophila* as a model to analyze homeostatic responses to stress. Sexually immature and sexually mature females and males from an isogenic wild-type strain raised under controlled environmental conditions were exposed to four

simple, highly reproducible, and high-throughput translatable stressors to facilitate the analysis of a large number of animals for direct comparisons. These animals were assessed in an open-field arena, in a light-dark box, and in a Forced Swim Test, as well as for sensitivity to the sedative effects of ethanol. These assays are a reliable index of general exploratory or escape behavior, and serve as indicators of centrally-mediated behavioral changes in response to a novel environment. The results establish that immature and mature females and males represent behaviorally distinct populations under control conditions as well as after exposure to different stressors. In addition, an adaptive response to a given stressor in one paradigm was not predictive for outcomes in other paradigms. Therefore, the neural substrates mediating the perception of, and response to, a stress must be differentially expressed depending upon the hormonal status of the brain. This work establishes *Drosophila* as a genetically tractable reductionist model to elucidate the molecular and cellular plasticity associated with the innate predilection of an organism to maintain neuronal homeostasis and maintain the appropriate stress responsivity.

574C

***princess glia,* a novel gene required for neuronal and cellular homeostasis. Wendi Neckameyer**[1], Davinelle Daniels[2], Andres Nieto[1], Amer Avdagic[1], Sarah Chan[1], Anisha Chava[1], Ryan Doan[1]. 1) Dept Pharmac & Physiol Sci, St Louis Univ School Med, St Louis, MO; 2) Academic Affairs, Harris-Stowe State University, St Louis MO.

We initiated a loss-of-function screen using a collection of lines derived from an isogenic strain displaying wild-type locomotor and other behaviors. Genetically identical sexually immature and mature females and males were raised under the same environmental conditions, and then subjected to four different high-throughput, translatable, and easily reproducible stressors (starvation, oxidative stress, sleep deprivation and social isolation) to facilitate the analysis of a large number of animals for direct comparisons. The animals were assessed for locomotor changes in an open field arena via a high-resolution tracking paradigm and compared with the corresponding parental control. The assessed parameters have valid physiological relevance since they have been shown in numerous studies to be affected by stress and affective disorders. Forty animals were screened for each population and control or experimental condition to permit statistically meaningful comparisons between the different genotypes. We uncovered a line in which sexually mature females displayed multiple significantly altered motor parameters in response to all four stressors; this behavioral phenotype was fully recapitulated when expression of this gene was reduced only in neurons. Immature males and females and mature males displayed stress responses similar to those of the parental line, suggesting we had identified a gene critical for neural plasticity whose expression was dependent on the hormonal status of the brain. Targeted knockdown of this gene in glial cells was lethal, and this previously uncharacterized candidate was renamed *princess glia* (*prig*). Additional experiments demonstrate that *prig* is active all cells which are or can become mitotically active, and functions to regulate cell proliferation and autophagy as a critical regulator of cellular homeostasis.

575A

Transposable element misexpression and neuronal decline. L. Prazak[1,2], W. Li[2,3], N. Chatterjee[2], S. Grüninger[4,5], L. Krug[5], D. Theodorou[6], J. Dubnau[2,5]. 1) Farmingdale State College, Farmingdale, NY; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 3) Graduate Program in Molecular and Cellular Biology, Stony Brook University, Stony Brook, NY; 4) Institute of Neuroinformatics, University of Zurich, 8057 Zurich, Switzerland; 5) Watson School of Biological Sciences, Cold Spring Harbor Laboratory; 6) Magistère de Génétique Graduate Program at Université Paris Diderot, Sorbonne Paris Cité. Transposable elements (TEs) are highly abundant mobile elements that make up a large fraction of most eukaryotic genomes. Retrotransposons, a subclass of TEs, make up about 40% of the human genome and 30% of the *Drosophila* genome. Recent work has shown that TEs are active in somatic tissue with a correlation between increased TE expression and neurodegenerative disorders such as amyotropic lateral sclerosis and frontotemporal lobar degeneration. Here we show transcripts from the *R2* and *gypsy* transposons are significantly elevated in head tissue during normal aging and we detect age-dependent expression of the *gypsy* membrane glycoprotein. Employing genetic manipulation techniques to disrupt the TE control mechanisms we show that flies harboring mutations in *Argonaute2*(d*Ago2)* result in accelerated age-dependent elevation of both *R2* and *gypsy* expression. A correlation between age-dependent neuronal decline and TE activation is evident as *dAgo2* mutants already exhibit a partial reduction in memory at 2-4-days old and this mild defect becomes dramatically worse in 20-day old mutant adults. It was also found that *dAgo2* mutant flies exhibited significantly shorter lifespans than their wild type counterparts. Furthermore, we have been able to detect *de novo* integration events using a *"gypsy-TRAP"* reporter system supporting the conclusion that *gypsy* not only is expressed in neurons of aging animals, but also is actively mobile. This reporter system was also used to quantify *de novo* gypsy insertions throughout the central brain. In this analysis, 130 of 220 detected insertions were found to be in mushroom body Kenyon Cells (KCs), a brain region critical for olfactory memory, despite the fact that KCs contribute only ~5% of the total neurons. These findings suggest that transposon activation may contribute to age-dependent loss of neuronal function. .

576B

Determination of environmental factors affecting social space in *Drosophila melanogaster*. Alison R. McNeil[1], Nicholas Choi[1], Adesanya A. Akinleye[2], Sam N. Jolley[1], Zulekha Rouzyi[2], **Anne F. Simon**[1]. 1) Department of Biology, Faculty of Science, Western Ontario University, Ontario, London, Canada; 2) Department of Biology, York College of the City University of New York, Jamaica, NY, USA.

In addition to genetic factors, several environmental factors contribute to social behaviour of *Drosophila melanogaster* seen within a social group. One way to quantify flies behaviour within a social group is to measure their individual social space. Social space refers to the distance between a fly and its nearest neighbour. For a given population of *D. melanogaster*, this measurement is stable when testing under the same conditions. For example, *Canton-S* flies create a reproducible social space distance that is within approximately two body lengths of each other. Therefore, social space can be used to study social interactions of flies within a group. Our lab had

developed the assay to quantify this metric in a straightforward manner, specifically to be used as a diagnostic tool to easily study this form of social behaviour. The purpose of the work presented here is to better understand the environmental factors that affect social space. This will allow researchers to predict which social behaviour variations can occur and why. To further investigate these variations, groups of *Canton-S* flies were used to understand the dynamic behind social space. Their social space was measured under various conditions using the same type of chamber. Unlike several other behaviours, our results show that gender of the flies has no effect on social space. However, the parameters of the chamber, including size and orientation (vertical and horizontal placement), do affect social space. Similarly, density of the group also affects social space. In addition, previous social isolation affects social space, as does the time of day when the interaction is tested. Knowledge of the variations created by these conditions will allow the social space assay to become a better diagnostic tool. It will give researchers a reliable measure to study social behaviour.

577C

Characterization of a novel bang-sensitive gene in *Drosophila melanogaster*. Ghazal Stity, Christopher J. Jones. Biological Sciences, Moravian College, Bethlehem, PA.

Fruit flies carrying "bang-sensitive" mutations display a seizure-like phenotype when exposed to vigorous physical shock such as banging their container on the benchtop. Relatively few mutations of this class have been identified and mapped to their respective genes, which is an essential step in further characterizing the roles of these genes in triggering the seizure phenotype and their effects on the flies' overall well-being. Understanding bang-sensitive mutations is particularly interesting for the promise they hold for influencing the way seizures in humans are viewed. Preliminary testing of a recently-acquired uncharacterized bang-sensitive mutant has confirmed that the mutation is on the X chromosome, and that it is not in any of the known X-linked bang-sensitive genes. Recombination, deletion, and duplication mapping were used to narrow the list of candidate genes to four. Experiments examining phenotypic characteristics such as temperature sensitivity, seizure and refractory period length, and longevity have been carried out. Comparison of these results with comparable data from known bang-sensitive mutants confirms that this is a novel member of this class of mutants. .

578A

The JAK-STAT signaling pathway functions in GABAergic neurons to mediate the effects of ethanol on developmental and adult behavior. Kimberly McClure, Gabriella Ceresa, Amanda Menzie, Axel Munoz, Gina Trotto. Biology, Elmhurst College, Elmhurst, IL.

Developmental ethanol exposure in flies can produce phenotypes reminiscent of Fetal Alcohool Syndrome (FAS) in humans, such as an increase in lethality, developmental delays and changes in adult behavior. Similarly, acute ethanol exposure in the adult fly triggers a series of behavioral changes that are strikingly similar to those seen in mammals, including an increase in activity followed by loss of postural control and eventual sedation. The mechanism(s) by which ethanol impacts the central nervous system (CNS) leading to ethanol-induced sedation and the genes modulated by ethanol during development remain largely unknown. Here we report that the JAK-STAT signaling pathway mediates both the effects of ethanol on development and adult behavior. Using genetic and behavioral analyses, we show that inhibition of the JAK-STAT pathway in neurons expressing the *vesicular GABA transporter* (*VGAT*) increases sensitivity to ethanol sedation, while activation of the pathway decreases ethanol sedation sensitivity. Similarly, pan-neuronal inhibition of the JAK-STAT pathway during developmental ethanol exposure increases ethanol-induced lethality, while activation of the pathway decreases ethanol-induced lethality. Additionally, we find that components of the JAK-STAT pathway are regulated in the adult fly CNS by acute ethanol exposure. We propose that the JAK-STAT pathway plays a critical role in mediating the response to ethanol both in the adult and developing fly.

579B

Effects of Ethanol Exposure on Development Time and Survival in Drosophila. Yasmeen N. Hussain[1,2], Victoria A. Pray[1], Eugenea V. Zhirnov[2], Morgan Davis[1], Rachel A. Lyman[1], Lenovia J. McCoy[1], Tatiana V. Morozova[1,2], Robert R. H. Anholt[1,2], Trudy F. C. Mackay[1,2]. 1) Department of Biological Sciences, North Carolina State University, NC; 2) W.M. Keck Center for Behavioral Biology, North Carolina State University.

Alcohol consumption during pregnancy can result in birth defects that comprise fetal alcohol syndrome. We used *Drosophila melanogaster* as a model to assess genetic risk factors that contribute to adverse effects during early exposure to ethanol. We used a collection of 201 inbred wild-derived lines with sequenced genomes from the *Drosophila melanogaster* Genetic Reference Panel (DGRP) to measure differences in development time and viability between flies reared on ethanol-supplemented medium and controls. To measure development time and viability, we placed 50 eggs from the same parental vials on standard fly food and on food supplemented with 10% ethanol. Development time was measured as the number of days it takes to develop from eggs to adult flies. We estimated survivorship as the percentage of eggs that developed to adult stage. The percentage of eggs that survived was lower on ethanol than on regular food. We found significant variation among the DGRP lines in survival on ethanol-supplemented food versus regular food. We also found significant differences in development time. For most lines, growth on ethanol-supplemented food caused an increase in development time. Since whole genome sequences for these lines are available, we performed genome-wide association analyses and identified polymorphisms in candidate genes associated with both traits. In addition we identified candidate genes contributing to variation in the difference between development time and survival on ethanol supplemented medium and regular medium (i.e. sensitivity). Contributions of candidate genes to ethanol sensitivity affecting development time and survival can be assessed through mutational analyses and targeted RNAi. Human orthologues can then be targeted as candidate risk factors for fetal alcohol syndrome. Supported by NIH grants AA016560.

580C

Analysis of the Pruritic (Itch) Response in *Drosophila melanogaster*. Ciny John, John Nambu. Department of Biological Sciences, Charles E. Schmidt College of Science, Florida Atlantic University, Jupiter, FL.

Pruritus is a condition in which a scratching response is evoked by an unpleasant sensation caused by a pruritogen, or itch inducing agent. In mammals, pruritogens stimulate unmyelinated nerve endings in the skin and mucosa causing pruritus signals to travel to the spinal lateral grey column via the spinothalamic tract, inducing the itch response and subsequent scratching behavior. Pruritogens known to induce itching include histamine, which is released during mast cell degranulation; compound 48/80, which induces histamine release; cowhage spicules, which signal a histamine-independent pathway and chloroquine, an antimalarial drug for which itch is a side effect. Other itch regulators include interleukins, protease activated receptors (PAR), transient receptor potential (TRP) receptors and opioids. Itch is a primary symptom in acute reactions resulting from stings/bites and in chronic reactions as in many skin diseases. However, in some patients, pruritus is a symptom of systemic diseases including metabolic disorders, haematological diseases, cancer and HIV/AIDS. It can also result from pharmacotherapy and neuropsychiatric disorders like anxiety, depression, schizophrenia and delusions of parasitosis, which can often result in severe cases of self-injurious behavior. Therefore, cost-efficient investigation of pruritus in an established genetic model is important. In current study, we are developing *Drosophila melanogaster* as a model organism for investigating pruritus. To analyze the effects of established pruritogens on the behavior of *Drosophila*, we are administering aerosolized pruritogens using two methods: a vacuum sealed vial and using an airbrush, allowing the flies take in the pruritogen through the cuticle and spiracles. Our results indicate that both Histamine and Compound 48/80 elicit a significant behavioral response in flies resulting in extended time spent grooming/scratching, compared to the control flies. However, Chloroquine and Cowhage do not appear to significantly affect the flies' grooming/scratching behavior. Based on our current findings, we conclude that *Drosophila* can indeed be used as a model organism for pruritus and that both vertebrates and invertebrates experience itch. .

581A

Effects of Lead Exposure on Development Time and Survival in Drosophila. Sarah E. McAdams[1], Lenovia J. McCoy[1], Yasmeen N. Hussain[1,2], Shanshan Zhou[1,2], Tatiana V. Morozova[1,2], Trudy F.C. Mackay[1,2], Robert R.H. Anholt[1,2]. 1) Department of Biological Sciences, North Carolina State University, Raleigh, NC; 2) W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC.

Heavy metal toxicity is a world-wide health problem. Lead exposure, especially, is of concern due to the adverse effects of low concentrations on cognitive development in children. We used Drosophila melanogaster as a powerful genetic model to assess genetic risk factors that contribute to adverse effects during early exposure to lead. We used a collection of 196 inbred wild-derived lines with sequenced genomes from the Drosophila melanogaster Genetic Reference Panel to measure differences in development time and viability between flies reared on lead-supplemented medium and controls. We placed 50 first instar larvae from the same parental vials on standard fly food and on food supplemented with 5mM lead acetate. Development time was measured as the number of days it takes to develop from first instar larvae to adult flies. We estimated survivorship as the percentage of larvae that developed to adult stage. We found significant variation among the lines in survival on lead-supplemented food versus regular food. We also found significant differences in development time. For most lines, growth on lead-supplemented food caused an increase in development time. We performed genome-wide association analyses and identified polymorphisms in candidate genes associated with both traits, as well as genes contributing to variation in the difference between development time and survival on lead supplemented medium and regular medium (i.e. sensitivity). Contributions of candidate genes that harbor polymorphisms associated with lead sensitivity can be assessed through mutational analyses and targeted RNAi. These studies provide insights into genome-environment interactions that determine susceptibility to lead neurotoxicity and identify candidate genes with human orthologues as targets for future translational studies in human populations. Supported by NIH grant R21-ES021719. Shanshan Zhou is supported by an institutional postdoctoral NRSA (5T32ES7046-34).

582B

***Drosophila* as a low complexity model for myeloproliferative neoplasms. Abigail M. Anderson**, Elizabeth Rodkin, Erika Bach. Biochemistry and Molecular Pharmacology, NYU School of Medicine, New York, NY.

Myeloproliferative neoplasms (MPNs) are a group of hematopoietic disorders characterized by the overproduction of mature myeloid cells, with the majority of patients presenting with an activating mutation within JAK2 ($JAK2^{V617F}$). Prior to the discovery of the activating JAK2 mutation in MPNs, a dominant, temperature-sensitive mutation in the *Drosophila* JAK, *hopscotch* (*hop*, $hop^{Tumerous\text{-}lethal\ (Tum\text{-}l)}$), was shown to cause an overproduction of myeloid-like blood cells, which aggregate and become melanized, referred to as melanotic tumors. We are conducting a genetic modifier screen for novel modifiers of $hop^{Tum\text{-}l}$-mediated melanotic tumorigenesis using the Bloomington Deficiency Kit. We have completed deficiency screening of the majority of the autosomes and have identified multiple modifying genomic regions, which have not been previously associated with JAK/STAT signaling. We are currently further mapping these modifiers using smaller deficiencies and single gene mutations. Novel modifiers identified from this screen may potentially be therapeutic targets for MPNs and other JAK/STAT-associated disorders. This work was supported by the MOTI institutional T32 training grant (T32 CA009161 (Levy)) and by a CTSI pilot project award (NIH/NCATS UL1 TR000038 (Cronstein, PI)).

583C

Identifying new modulators of blood cell development using *Drosophila* as a low complexity model of human myeloproliferative neoplams. Alessandro A. Bailetti, Abigail Anderson, Erika Bach. Biochemistry and Molecular Pharmacology, New

York University School of Medicine , New York, NY.

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that cause over-proliferation of specific myeloid lineages. More than 96% of patients that present with one type of MPN called polycythemia vera have a point mutation in the *JAK2* gene. This mutation (*JAK2*^{V617F}) generates a constitutively active JAK2 protein that hyper-actives the JAK/STAT signaling pathway. Unlike vertebrates, which have four JAKs, *Drosophila* has a single JAK called Hopscotch (Hop). More than 30 years ago, it was reported that a dominant, temperature-sensitive allele of *hop* called*hop*$^{Tum-l}$ significantly increases the number of myeloid-like cells, similar to patients with the *JAK2*^{V617F} mutation, and causes melanotic tumors. The *hop*$^{Tum-l}$ tumor phenotype is dependent on activation of the JAK/STAT pathway, and reducing one copy of *Stat92E* in *hop*$^{Tum-l}$ flies strongly suppresses the tumor burden. Based on this dominant modification of the tumor burden by reduction of *Stat92E*, we hypothesize that reducing the dose of genes involved in JAK/STAT signaling or in hematopoiesis will enhance or suppress the tumor incidence or burden in *hop*$^{Tum-l}$ flies. Here we present the results from an F1 deficiency screen of the right arm of the second chromosome (2R). We are screening 92 deficiencies in the Bloomington kit, which together uncover 98.5% of the euchromatin on 2R. We use the "tumor burden" scoring system developed by the Li lab (Shi et al., Nature Genetics, 2006). If a deficiency modified the *hop*$^{Tum-l}$ phenotype more than one standard deviation away from the control mean (i.e. that of *hop*$^{Tum-l/+}$ alone), we scored it as an enhancer or suppressor. We will use smaller deficiencies to define the smallest genomic region that recapitulates the behavior of the original deficiency. Finally, we will characterize a subset of these candidate genes in the context of blood and lymph gland development. This work was supported in part by the Jack Kent Cooke Foundation Continuing Graduate Scholarship and the NSF Graduate Research Fellowship Program. .

584A

The role of RecQ proteins, BLM and WRNexo, in DNA repair, aging, and tumorigenesis. Elyse Bolterstein[1], Molly Ahern[2], Rob Salomon[3], Mitch McVey[2]. 1) Dept of Biology, Northeastern Illinois University, Chicago, IL; 2) Dept of Biology, Tufts University, Medford, MA; 3) Tufts University, School of Medicine, Boston, MA.

Members of the RecQ family of helicases are known as the "guardians of the genome" due to their essential roles in DNA repair, replication, and recombination. Mutations in the RecQ helicases, WRN and BLM, cause Werner and Bloom Syndrome – autosomal recessive diseases characterized by patients' increased risk of cancer and early onset of aging. While WRN contains both a helicase and exonuclease domain, the *Drosophila* homolog, WRNexo, contains only the exonuclease portion of WRN, providing us with a unique model system to study the functions of this protein. Similar to *Blm*, *WRNexo* mutants exhibit shorter life spans and greater tumor incidence, specifically affecting gut and germline cells. Interestingly, *WRNexo* males exhibit a higher incidence of testes tumors in comparison to *Blm* mutants. However, this greater likelihood of developing testes tumors has no effect on male fertility. *WRNexo* mutants also show physiological signs of early aging compared to wild type controls, including muscular degeneration and oenocyte pigmentation. Additionally, we observed increased antioxidant activity in *Blm* flies, suggesting that these characteristics may be due to an increase in oxidative damage. We have recently reported a likely interaction between WRNexo and BLM in response to replication stress. We also suggested that unlike BLM, WRNexo does not appear to play a role in homologous recombination (HR)-mediated repair of double-strand breaks (DSB). To test if WRNexo is important in DSB repair through end-joining (EJ), we conducted a *P{w*^{a}*}* excision assay in the absence of HR. Though EJ repair products in *WRNexo* progeny did not differ from wild type controls, this assay largely investigates repair involving alternative-EJ and not classical non-homologous EJ (C-NHEJ), which has been previously linked to WRN. Therefore, future experiments will focus on the role of WRNexo specifically in (C-NHEJ).

585B

Comparative genomics of Drosophila melanogaster and a butterfly, Cercyonis pegala, to model metastatic melanoma. Thomas Brown. Thomas M. Brown, Ph.D., President, Genectar Com LLC, 539 Kalispell Ave. Whitefish, MT 59937.

Melanoma at transition to stage III (metastasis) is a cancer with high case fatalities. Similarities exist with development of melanic cells; e.g., mobility of the cells. Comparative genomics in insect models including Drosophila might lead to new approaches or pharmaceuticals to defeat metastatic melanoma, hence this study. Sophisticated genomics of Drosophila spp. and mosquitoes has included detailed analyses of development of the wing imaginal disc. With genome projects of three butterflies, Bombyx mori, the mulberry silkworm, (Lepidoptera: Bombycidae) and several other lepidopterans, the opportunity exists to find candidate genes specific to the complex patterns of pigmentation in the wing. Genectar has colonized a lesser studied butterfly which is native to Montana, Cercyonis pegala. This butterfly possesses eyespots on the wing of the adult very similar to those of Bicyclus anynana for which genes controlling the pattern development have been elucidated. A comparative genomics approach holds promise. Whole genome sequences were search from alignment with relatively conserved portions of known mammalian genes using Blast. Identified matches where translated via BioX and continued outward from the whole genome sequence and linked by alignment with the originally searched gene. A new ABC1-like gene was mined from the whole genome sequence of the southern house mosquito, Culex pipiens quinquefasciatus (Diptera: Culicidae). A partial repeat of the new gene was found approximated 2kb upstream in the same reading frame. This observation will be useful in understanding gene duplication among the ABCA genes and the large superfamilies of ABC proteins. Sequences found in honey bee and rust red flour beetle and were useful in finding the new sequence. A new golden-like gene was found from the whole genome sequences of B. mori. This inferred amino acid sequence was more similar to human SLC24A2 retinal cone sodium-calcium potassium exchanger than it was to golden-like SLC24A5. This observation will be useful in understanding the large superfamilies of SLC proteins. To our knowledge an arthropod golden-like gene has not been annotated at this time. An insect sequence was similar to the BTB domain of NAC1 oncogene. .

586C

Oncogene-specific effects of abnormal systemic metabolism on tumor growth and metastasis. Kiu Ming April Kong, Lisa Shim, Cemre Cetin, Arthur Hilliker, Spencer Mukai, Kyle Belozerov. Department of Biology, York University, Toronto, Canada.

A large number of epidemiologic studies suggest a link between metabolic diseases, such as obesity and type 2 diabetes, and the aggressiveness of certain tumor types. For instance, enhanced lymph node metastasis was documented in obese breast cancer patients with progesterone receptor mutations. Despite these clear epidemiologic associations, little is known about the specific molecular mechanisms linking cancer progression and metabolic dysfunction. As dietary manipulations in *Drosophila* induce metabolic states similar to human diseases, such as hyperglycemia, insulin resistance, and accumulation of body fat, flies offer an excellent system for examining these mechanistic connections. We generated larvae carrying oncogenically transformed eye imaginal disc epithelia, and examined the progression of these tumors in animals fed a variety of macronutrient regimens. Striking changes in the overall tumor volume and the number of individual micro-metastases were observed in animals fed high-sugar diet, and to a lesser extent high-protein and fat diets. Importantly, the type of oncogenic transformation was found to be a critical determinant of tumor response to dietary manipulations, suggesting that systemic hyperglycemia and hyperinsulinemia exert context-dependent effects on tumor progression. For instance, tumors driven by the activated *Notch* pathway are exquisitely sensitive to hyperglycemic state, whereas tumors containing aberrations in the *Hippo* pathway appear significantly less affected by high-sugar diet. A molecular model consistent with the observed differences will be presented, and the applicability of our *in vivo* assay to studying metabolic sensitivity of specific oncogenic signatures from human cancers will be discussed. .

587A

***TRIM3*, a human ortholog of *Drosophila brat,* maintains asymmetric cell division of glioma stem cells by regulating NOTCH1 transport. Subhas Mukherjee**[1], Jun Kong[2], Gang Chen[1], Daniel Brat[1,2]. 1) Pathology and Laboratory Medicine, Winship Cancer Institute of Emory University, Atlanta, GA; 2) Department of Bioinformatics, Emory University, Atlanta, GA.

Glioma stem cells, capable of self-renewal and multipotent differentiation, influence neoplastic growth by a dynamic balance between symmetric and asymmetric cell division. Growth is favored by a deregulation of asymmetric division in which greater numbers of self-renewing cells emerge, as opposed to one stem and one differentiated cell. *Drosophila* Brain Tumor (Brat) protein is a critical driver of asymmetric division and differentiation, and neuroblast cells devoid of Brat undergo symmetric cell division to generate self-renewing cells that result in the *brat* mutant phenotype. Using a *brat* RNAi driven by the neuroblast specific promoter *inscuteable* in *Drosophila* model, we demonstrated accumulation of proliferative neuroblasts marked by RFP. Reduced Brat in these settings was associated with upregulation of Notch protein, suggesting a suppressive role on Notch signaling by Brat, although detailed mechanisms are lacking. We have shown that *TRIM3*, a human ortholog of Drosophila *brat*, is deleted in more than 25% of glioblastomas (GBMs). We have demonstrated that expression of TRIM3 in GBM-derived neurospheres significantly reduces proliferation, neurosphere formation, and the expression of stem cell markers CD133, NESTIN and NANOG. In GBM stem cells, expression of TRIM3 leads to a greater percentage undergoing asymmetric cell division. We provide evidence that TRIM3 is attenuating early endosome formation thus affecting the packaging of active NOTCH1 (NICD) into endosome. This restricts transport of NICD into nucleus, inhibiting NOTCH1 mediated proliferation and self-renewal. Altogether, our current data support a critical role of TRIM3 in maintaining asymmetric cell division of glioma stem cells by regulating NOTCH1 transport. .

588B

Role for SETDB1 in maintaining blood cell homeostasis in the *Drosophila* larvae. Indira Paddibhatla. Biol Dept, Center for Cellular and Molecular Biology, Hyderabad, India.

Epigenetic mechanisms and their role in growth and development of cancer are emerging to be very significant. SETBD1/Eggless catalyzes the epigenetic mark of lysine 9 methylation in Histone 3. In 70% of melanomas this enzyme is shown to be upregulated. Using fruit flies we provide insights into role of SETDB1 in maintaining quiescence of hematopoietic cells. In this study we characterized the role of dSETDB1 in blood cell growth and differentiation in *Drosophila melanogaster*. In hemolymph and lymph gland of third instar larvae there are undifferentiated precursor blood cells (yet to encounter signals for maturation) and matured blood cells. To date three matured cell types are characterized; plasmatocytes (>95%), crystal cells (<5%) and lamellocytes (<1%). Loss of function mutants of SETDB1 showed hematopoietic defects; increased proliferation and differentiation of blood cells into lamellocytes, decreased crystal cells along with melanotic microtumor formation. To examine the involvement of the niche cells referred as posterior signalling center we looked into the expression of Antennapedia that is normally expressed in the niche of lymph gland. We noticed that the hemocytes expressing Antp were increased in loss of function mutants of SETDB1. We next elucidated the effects of misexpression of SETDB1 both in circulation and cortical zone (specifically in the Lozenge positive precussors and matured crystal cells) of the lymph glands. Our results showed increased number of crystal cells with a simultaneous increase in the Notch-ICD expression in the anterior lobes of the lymph glands. Together our data indicates an involvement for SETDB1/Eggless in hematopoiesis of third instar *Drosophila melanogaster* larvae involving the maintainance of blood cell homeostasis in the lymph glands.

589C

JAK/STAT pathway control of cell competition during development. Poojitha Sitaram, Erika Bach. Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY.

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is the primary mechanism of cytokine and growth factor signaling in mammals. Dysregulated JAK/STAT signaling results in various human cancers. Roles of the JAK/STAT pathway

in growth control have been well described in *Drosophila*. The *Drosophila* JAK/STAT pathway is simpler than its vertebrate counterparts. It has three IL-6-like cytokines, one gp130-like receptor, one JAK tyrosine kinase Hopscotch and one STAT transcription factor. Our lab reported a novel role of the *Drosophila* JAK/STAT pathway in a cellular phenomenon termed cell competition, which regulates tissue size and quality control during development. Cell competition is induced along the interface of adjacent viable cell populations that differ in metabolic rates: cells with higher metabolic rates (called winners) cause the apoptotic death of cells with lower rates (called losers). Several conserved factors - including Myc and Wnt - have been identified as key regulators of cell competition. Some winners - particularly those with increased Myc - out-compete wild-type cells located several cell diameters away and have been termed supercompetitors. Cell competition regulates tissue size and shares properties with the early stages of cancer. It also evolutionarily conserved exists in mammals, but it has been best studied in the *Drosophila*wing. Our lab is interested in elucidating the role of the JAK/STAT pathway in cell competition. We have previously demonstrated that JAK/STAT signaling controls cell competition independently of Myc and Wnt. We find that cells lacking STAT become losers and are eliminated by their healthy neighbors. By contrast, cells with sustained Stat92E activation become supercompetitiors through an unknown mechanism. In order to identify the mechanism by which the activation of this pathway in a subset of cells confers supercompetitor status, leading to the elimination of neighboring losers, we will perform genome-wide expression profiling to identify genes upregulated in JAK/STAT supecompetitors. We will further characterize a select few of the genes identified to determine their role in JAK/STAT-mediated cell competition.

590A

Systemic Effects of Tissue Overgrowth on Hematopoiesis. Carrie Spratford, Banerjee Utpal. Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA.

Understanding how tumors communicate with neighboring healthy tissues within the same organ is a topic that has been well studied in recent years. However, less understood is how hyperproliferative cells or tumors in one organ communicate with cells in other organs to alter gene expression, metabolism, or state of differentiation. We have initiated exploring this issue by developing a genetic background that directly causes hyperproliferation of the epithelial tissues, while non-autonomously causing the premature differentiation of progenitors within the larval lymph gland (LG). The LG is a main contributor for hematopoiesis throughout larval development and is made up of three distinct zones; the posterior signaling center (PSC), the medullary zone, and the cortical zone. It is well known that the blood progenitors in the medullary zone are maintained by signals emanating from both the PSC and the cortical zone. In our system, tissue hyperproliferation is achieved by the specific expression of an activated form of the*Drosophila* PDGF/VEGF-related receptor (Pvr^{ACT}) in cells of the imaginal disc epithelium. The resulting tumor systemically causes extensive premature differentiation of the LG precursors, a spongy morphology throughout the LG, as well as the accumulation of a thick shell-like extracellular matrix. This distinct phenotype suggests that the presence of a tumor produces systemic changes to the animal including the altered development of the hematopoietic system. Our lab produced RNAseq data identifying several secreted candidate molecules that may mediate this phenotype. Targeted over-expression and RNAi knock down is being utilized in order to test candidates for requirement and sufficiency in induction of the differentiation phenotype. *Drosophila* is an ideal model system in which to study the relationship between tumor burden and hematopoiesis as the genome is much simpler than vertebrate systems and there are many powerful genetic tools that can modulate gene expression in specific organs or cell types. Once identified, the pathway will be studied in the context of normal development to understand how epithelial tissues normally communicate with the LG to regulate blood cell differentiation.

591B

Studying a model of Autism Spectrum Disorder in *Drosophila*. Marlene Cassar, Doris Kretzschmar. Oregon Institute of Occupational Health Sciences, OHSU, Portland, OR.

Although originally be thought to be environmentally induced, genetic causes are now well established in Autism Spectrum Disorder (ASD). Most of the mutations identified in patients diagnosed with ASD are involved in neural processes such as neurogenesis (Homeobox A1), synapse function (Neurogilin 3), neuronal cell adhesion function and neuronal activity regulation (UBE3A and MECP2). These genes have also been linked to syndromes of ASD like Angelman Syndrome (UBE3A) and Rett Syndrome (MECP2). Ubiquitination has been implicated in ASD pathogenesis, as well as in other disorders of the nervous system like Parkinson's and Alzheimer's diseases, with mutations in genes like UBE3A, Parkin and UBB^{+1}. Researchers at OHSU have recently identified a new mutation in a family with several children displaying ASD. This protein is another ubiquitin ligase called HECW1 and we are now studying the role of this protein in behaviour in the fly model. In order to investigate HECW1 function in the*Drosophila* brain, we decided to use a pan-neuronal knock-down of HECW1 via RNA interference and focused on several behavioural assays: the fast phototaxis assay (locomotion and vision), the courtship assay (social interaction), the bang sensitivity assay (seizure) and tracking of freely moving flies (locomotion and activity). We found that pan-neuronal inhibition of HECW1 increased sensitivity to induced seizure, induced abnormal courtship behaviour and caused hyperactivity when compared to controls. Flies did not exhibit any visual or locomotion defects. Interestingly, we found an effect of gender with males being more affected than females. We also analyzed brains by histological and immunohistochemical means but were not able to find any structural defects either in males or females when compared to controls. These results strongly suggest that the behavioural defects are not due to severe structural defects. In summary, our results suggest that our model is able to recapitulate key features of ASD symptoms like social impairments, hyperactivity and sensitivity to seizure, and similar to human patients males are more affected. We are now using this new *Drosophila* model to get more insights into the role of ubiquitination in ASD.

592C

Developmental ethanol exposure disrupts lipid metabolism and causes oxidative stress in *Drosophila* larvae. Payam Khodabakhshi, Theresa Logan-Garbisch, Tony Bortolazzo, Peter Luu, David Do, Rachael French. San Jose State University, San Jose, CA.

Fetal Alcohol Spectrum Disorder (FASD) is a collection of physiological and behavioral abnormalities caused by maternal consumption of alcohol during pregnancy. Despite decades of research, the targets of developmental ethanol exposure in mammals remain elusive. We have established a genetic model of developmental alcohol exposure (DAE) in *Drosophila melanogaster* that mimics the detrimental effects of FASD in mammals, allowing us to better understand the molecular mechanism of FASD. We have previously shown that DAE leads to reduced expression of *Drosophila* insulin-like peptides (dILPs) and their receptor (InR). Here, we report on our recent work linking this observation to dysregulation of fatty acid metabolism. Further, through genetic analysis of mutant strains defective in the response to oxidative stress as well as pharmacological manipulation of the levels of reactive oxygen species, we have shown that DAE in *Drosophila* causes oxidative stress and that this stress is a primary cause of the lethality and developmental delay associated with DAE. In addition, using microscopy, gene expression analysis, and genetic screening, we show that DAE causes lipid accumulation, which is one of the causes of ethanol-induced oxidative stress. These data suggest a previously uncharacterized mechanism by which ethanol causes the symptoms associated with FASD. We will present the results described above, as well as the results of our current experiments to alter developmental ethanol sensitivity through dietary changes, specifically manipulation of the level and types of fatty acids and sugars in the animals' diets. This research was supported by a grant from the NIH National Institute of General Medical Sciences (5SC3GM103739).

593A

The ANKLE2 and VRK1 pathway: microcephaly in flies and humans. Nichole Link, Wu-Lin Charng, Jim Lupski, Hugo Bellen. Baylor College of Medicine, Houston, TX.

Microcephaly, or reduced brain size, is often the result of a neurodevelopmental disease with associated cognitive and neurological defects. In a forward genetic screen designed to identify genes causing neurodegeneration or neurodevelopmental phenotypes, we identified a mutation in *l(1)G0222* or *dANKLE2* (*Drosophila* ankryin repeat and LEM domain containing 2), which results in a small brain phenotype. To find a possible link between the human homolog, *ANKLE2*, and human disease, we surveyed the exome database of the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) for mutations linked to rare human Mendelian disorders. Using this strategy, we identified compound heterozygous mutations in *ANKLE2* in two patients that exhibit severe microcephaly as well as cognitive and neurological defects. Interestingly, our data suggest that loss of *dANKLE2* in *Drosophila* causes cell loss in the central nervous system, mimicking microcephaly phenotypes found in humans. *dANKLE2*mutants also exhibit defects in the peripheral nervous system and contain fewer neuronal stem cells that divide less frequently. Our data show potent genetic interactions between *VRK1* (*Ballchen* in*Drosophila*) and *dANKLE2*. Interestingly, mutations in *VRK1* have also recently been shown to cause microcephaly in humans. We argue that this novel pathway intersects with players that determine apico-basal polarity.

594B

Using *Drosophila* to characterise the human disease gene *MEGF8*. Deborah Lloyd, Andrew Wilkie, Tudor Fulga. Weatherall Institute of Molecular Medicine, University of Oxford, United Kingdom.

Mutations in the gene *Multiple Epidermal-Growth-Factor-like-domains 8* (*MEGF8*) result in a subtype of the congenital disorder Carpenter syndrome, more commonly caused by mutations in *RAB23*.The clinical features, which include craniosynostosis (premature fusion of the cranial sutures), polysyndactyly of the hands and feet, and laterality defects, are consistent with an essential role for MEGF8 in left-right patterning along with limb and cranial suture development. MEGF8 is a highly conserved protein with deep evolutionary origins yet little is known about its function other than proposed roles in nodal and BMP signaling. Here we describe our use of *Drosophila* as a model system to investigate the cell biology and function of MEGF8. Using the *Gal4-UAS* system, we performed targeted *in vivo* RNAi knockdown of the *Drosophila MEGF8* orthologue *CG7466,* which resulted in lethality with the ubiquitous drivers *Tubulin-Gal4*(100%) and *Actin5c-Gal4* (94%) along with the muscle-driver *24B-Gal4* (82%) and the wing-driver *Sal-Gal4* (74%). Furthermore, ~50% survivors from the *Actin5c-Gal4* cross had bristle defects reminiscent of phenotypes in planar cell polarity (PCP) fly mutants; interestingly, similar PCP-type defects are also seen in *Rab23* mutant flies. These phenotypes indicate an important role for *CG7466* in fly development, potentially functioning in PCP. We have employed various state-of-the-art approaches to generate a range of genetic and molecular reagents for the *Drosophila MEGF8* orthologue in order to comprehensively investigate its function. For example, we have developed a Golden Gate-based cloning strategy to assemble the *CG7466* gDNA (>10kb) and combined this with Gateway Recombination to develop a transgenic overexpression construct. We also took advantage of the versatility of CRISPR-Cas9 genome engineering to rapidly generate several mutant variants of this gene. These reagents will allow us to decipher the precise cellular function of this protein, thus contributing towards unravelling the molecular mechanisms underlying the human disorder and furthering our understanding of developmental processes in cranial suture homeostasis and patterning/asymmetry.

595C

Diminished MTORC1-Dependent JNK-Activation Underlies the Neurodevelopmental Defects Associated with Lysosomal Dysfunction. Ching-On Wong[1], Michela Palmieri[2], Dmitry Akhmedov[1], Jiaxing Li[3], Yufang Chao[1], Geoffrey Broadhead[1], Catherine Collins[3], Rebecca Berdeaux[1], Marco Sardiello[2], **Kartik Venkatachalam**[1,4,5]. 1) Integrative Biology and Pharmacology, University of Texas, School of Medicine, Houston, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, Texas, TX 77030; 3) Department of Molecular, Cellular and

Developmental Biology, University of Michigan, Ann Arbor, MI 48109; 4) Program in Cell and Regulatory Biology (CRB), Graduate School of Biomedical Sciences, University of Texas School of Medicine, Houston, TX 77030; 5) Program in Neuroscience, Graduate School of Biomedical Sciences, University of Texas School of Medicine, Houston, TX 77030.

Lysosomal storage diseases (LSDs) often lead to neurodevelopmental defects of unknown etiology. Here, we evaluated the mechanistic basis of these defects in *Drosophila* models of Mucolipidosis type IV and Batten disease. We found that lysosomal dysfunction, which characterizes these models, resulted in pupal lethality and fewer synaptic boutons at larval neuromuscular junctions (NMJs) owing to subthreshold activation of Rag GTPases and Mechanistic Target of Rapamycin Complex 1 (MTORC1). Involvement of MTORC1 in synaptic development was unexpected because NMJ bouton numbers are independent of S6K and 4E-BP1. Rather, MTORC1 promotes synaptic growth via JNK. Consistent with our findings in *Drosophila*, neurons in a mouse Batten disease model also exhibited diminished JNK activation. Despite decreased activation of an amino acid-responsive cascade involving MTORC1, the observed synaptic defects were surprisingly not suppressed by elevating dietary protein levels. This paradoxical finding was explained by the action of Anaplastic Lymphoma Kinase (ALK), which restricts neuronal amino acid uptake, because simultaneous administration of a high protein diet and an ALK inhibitor significantly suppressed the synaptic defects and pupal lethality associated with lysosomal dysfunction. In summary, we provide a novel explanation for the neurodevelopmental deficits following lysosomal dysfunction and suggest a pharmacotherapeutic strategy to ameliorate these defects.

596A

Characterization of the *Drosophila* insulin-signaling pathway. Laura Musselman[1], Thomas Baranski[2]. 1) Biological Sciences, Binghamton University, State University of New York, Binghamton, NY; 2) Washington University School of Medicine, St. Louis, MO.

Insulin resistance in type 2 diabetics is associated with lipotoxicity, cardiovascular disease, inflammation, blindness, peripheral neuropathy, non-alcoholic fatty liver disease, and obesity. We are interested in the downstream mediators of insulin signaling that contribute to this broad range of pathophysiological consequences. Loss- and gain-of-function genetics were used to identify differentially-expressed genes that respond to increased (constitutively active) or decreased insulin receptor (InR) activity. More than 120 genes were reciprocally expressed in the fat bodies of *Drosophila* larvae carrying these InR mutations. Interestingly, genes encoding proteins reported to function in the response to infection were over-represented in both datasets. We are testing the roles of genes involved in the immune response to see whether they affect insulin signaling in the fat body. These studies will enrich our understanding of the connections between inflammation and metabolic disease. .

597B

QTL candidate gene expression correlation with diet-specific glucose levels. Jaron N Nix, Laura K Reed. The University of Alabama, Tuscaloosa, AL.

An organism's phenotype is the product of its genotype and environment. Drosophila melanogaster, an organism with an evolutionarily conserved genetic similarity to humans, is a good model organism in which this genotype and environmental interaction can be better understood. We have identified diet-specific QTLs for glucose levels in the flies. Using RT-PCR, we characterized the expression levels of seven genes judged to be strong candidates for being causal of their corresponding QTLs. We were able to correlate the diet-specific glucose levels on either a high fat or normal diet with the expression levels of several of these genes. Thus, several of these candidate genes are likely to play a functional role in glucose homeostasis. Functional characterization and confirmation of these candidates genes is presently underway.

598C

Metabolic impact of maternal obesity on offspring. Rita Brookheart, Christina Collins, Laura Cline, Jennifer Duncan. Pediatrics, Washington University in St. Louis, St. Louis, MO.

A quarter of all pregnant women in the U.S. are obese and at high risk for miscarriage and preeclampsia, while their offspring are susceptible to developmental anomalies and long-term metabolic complications. The mechanism by which maternal diet impacts offspring health is unclear. Studies in animal model show a correlation between maternal obesity and diabetes and altered mitochondria in ovaries and offspring. Based on these observations and because mitochondria are maternally inherited, we hypothesize that mitochondrial metabolic programming is dependent on maternal diet and occurs early in offspring development.
To test this hypothesis, we utilized a *Drosophila melanogaster* model of maternal obesity in which w^{1118} virgin females are exposed to high sucrose (HSD) or low sucrose diets (LSD) for 7 days and then crossed with w^{1118} males. We have demonstrated that HSD females exhibit an obesity phenotype while offspring show changes in body composition, metabolic gene expression, and circulating sugars, all indicative of altered metabolism. In the present study, we characterized HSD and LSD female and offspring mitochondria. Mitochondrial number was altered in HSD female ovaries and offspring, based on mitochondrial DNA content. We examined mitochondrial function using a fly line expressing a dual-fluorescence ATP:ADP reporter gene. ATP and ADP levels and ratio were significantly decreased in HSD female ovaries. HSD offspring also exhibited decreased ATP:ADP. We next measured mitochondrial gene expression by qPCR and observed significant changes in the gene expression profiles of HSD female ovaries and their offspring. Together these data indicate that maternal caloric excess disrupts ovarian mitochondria and contributes to offspring mitochondrial dysfunction. To explore the mechanism behind this observation, we assessed the contribution of insulin signaling, which is concomitant with mitochondrial dysfunction. We demonstrate that HSD females are insulin resistant and have altered *dILP*expression, suggesting a role for maternal insulin signaling in offspring mitochondrial function. Ongoing studies focus on how maternal insulin signaling may contribute to diet-induced mitochondrial dysfunction in offspring.

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599A

Lipid droplet flux in the larval midgut of Drosophila. Ron Dubreuil, Bianca Diaconeasa, Jamie Moy, Sneha Gummuluri. Dept Biological Sci, Univ Illinois, Chicago, IL.

There is currently great interest in the mechanisms of normal lipid droplet biogenesis and the abnormal lipid droplet accumulation seen in human diseases such as hepatic steatosis and obesity. The major lipid carrier of dietary lipids from the midgut to the larval fat body in *Drosophila* is lipophorin (Lpp). Lpp apoprotein is related to ApoB48 which plays a comparable role in mammals. However, unlike ApoB48, which is produced in enterocytes, Lpp is initially produced in larval fat body). But ultimately Lpp traffics to the digestive tract where lipid loading takes place. Previous studies showed that loss of lipophorin pathway function leads to a striking abnormal accumulation of lipid droplets in the midgut. Here we examined the relationships between lipid droplet formation and lipophorin loading under a number of conditions. By Oil Red O staining relatively few lipid droplets accumulated in enterocytes of control larvae. These lipid droplets were short-lived, disappearing during a brief chase period with no food. Feeding larvae a high-fat diet (yeast paste supplemented with 10% oleic acid) led to massive lipid droplet formation in enterocytes. Apparently the capacity for fatty acid uptake can outpace Lpp loading and transport. Nevertheless, lipid droplets were cleared during a 24 hour chase without added oleic acid. In contrast, food oils (olive oil, canola, peanut, fast food hamburgers, etc.) did not produce the dramatic lipid droplet accumulation seen with oleic acid, unless also supplemented with porcine pancreatic lipase. Thus, while enterocytes are capable of robust fatty acid uptake, the gut lumen (with its endogenous lipase activity) is far less efficient at generating free fatty acids from dietary triglycerides. In long-term feeding experiments oleic acid was toxic resulting in a significant developmental delay and lethality. Thus the observed mismatch between fatty acid generation and absorption may be a way to avoid lipotoxicity.

600B

An investigation of the effects of HFD in the head of *Drosophila melanogaster*. Osvaldo Rivera, Jamon Harris, Silvienne Sint Jago, Siddhartha Dhakal, Matthew Talbert. Biology, University of Louisiana at Monroe, Monroe, LA.

Obesity predisposes individuals to a range of life-threatening comorbidities, including type 2 diabetes and cardiovascular disease, while also increasing mortality directly. Proper energy homeostasis is dependent on normal brain function, which integrates peripheral signals, modulates autonomic outflow and controls feeding behavior. Candidate obesity genes are enriched for neurological function in humans, and obesity may predispose individuals to neurological disease, such as Alzheimer's. When *Drosophila melanogaster* (flies) are exposed to high fat diet (HFD) by supplementing a standard cornmeal-sucrose-yeast medium with coconut oil, a rich source of saturated fat, they adopt an obese phenotype that results in decreased lifespan, increased triglyceride storage, and hindered climbing ability, the latter of which often indicates neurological decline. Our objective is to establish the obesity-like phenotype and determine a correlation, if any, between obesity and neuropathology in flies through behavioral and genetic analysis. To induce a an obese phenotype, synchronously mated female w^{1118} flies (Bloomington labs) were placed on a HFD media containing cornmeal (5.2%), sucrose (10%), yeast (10%) and molecular grade coconut oil (20%), with control flies exempt from any coconut oil. Flies were examined at 7, 14, and 21 days of exposure to the experimental diets. Flies maintained an obese phenotype through adult life with detectable onset occurring in 7 days as evidenced by increased triglyceride stores and diminished climbing ability. HFD exposure also resulted in a significant reduction in lifespan. To further our study, a Drosophila Genome Array 2.0 (Affymetrix) was carried out. Although similar studies have examined the transcriptome of whole flies exposed to a HFD, none have yet shown such data from fly heads. Due to elimination of background, the genomic response to HFD in the fly head could reveal novel mechanisms of neural or body-wide pathology.

601C

Metabolic Analysis of IDH Mutant Gliomas in *Drosophila* . M. Brown, J. Buccetti, J. Mills, M. Tipping. Biology, Providence College, Providence, RI.

Metabolic reprogramming is a common hallmark shared by nearly all proliferating cancer cells, and thus has emerged as an exciting new direction in cancer research. Many signaling pathways have been implicated in mechanisms leading to the shift of metabolic programs in tumors, but more recently a small number of metabolic enzymes have also been identified in this process. Genes encoding the metabolic enzymes Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) were found to be mutated in up to 70% of low-grade and medium grade gliomas, and in 15-20% of adult acute leukemia samples. These findings were the first to link the IDH gene to tumorigenesis. IDH1 and IDH2 function to irreversibly catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). Although these are important metabolic enzymes, little is known about the metabolic impact on cells harboring mutant IDH proteins. Our goal is to model the IDH mutant phenotype in *Drosophila* glial cells for further characterization of both metabolic status, and IDH enzymatic activity. To this end, we have generated transformant flies that express the most commonly identified IDH mutation under control of the UAS-Gal4 system. We have also analyzed the metabolic flux of *Drosophila* cell lines with IDH mutations, IDH overexpression, and loss of IDH function using the Seahorse Bioscience XFe96 metabolic analyzer. Future plans include similar metabolic analysis on IDH transformant adult brains, as well as metabolic flux analysis (MFA) using labeled metabolites. The results of these investigations will identify potential new targets for the treatment of these aggressive tumors at the level of cellular metabolism.

602A

Effects of dim light at night through disruption of circadian rhythm on metabolism in *Drosophila melanogester*. Mary Kim[1], Su-yeon Yu[1], Yunjeong Kim[1], Hyo Sun Lee[1], Eunil Lee[1], Joong-Jean Park[2]. 1) Department of Preventive Medicine,College of Medicine, Korea University, Seoul, Korea; 2) Department of Preventive Phyology, College of Medicine,Korea University, Seoul, Korea.

Circadian rhythm is approximately 24-hour cycle present in every eukaryotic cells in both animal and plant. It is closely related with diverse biological process including locomotor activity, sleep, liver metabolism, blood pressure, body temperature and hormone secretion. Disruption of circadian rhythm is reported to increase cancers and metabolic disorders. Since only a few studies have been conducted on metabolic disorders by light pollution, there is a need for further study on the mechanisms of metabolic disorders. Exposure to light at night causes disruption of circadian rhythm and sleep disorder. We studied how dim light at night changes gene expression patterns in Drosophila. Flies were cultured at a 12:12 h light/dark cycle and exposed to dim light (10 lux) at the dark cycle for 3 days. Their locomotor activity increased temporarily when light was on or off. However, that of the flies exposed to dim light at night was extended for a few hours, even after the light was off. We analyzed the profiles of the total mRNAs from the fly heads 4 h before light was on or off with the genome-wide microarray chip. Interestingly, above 30% of the genes that showed more than 2 times of change in expression belonged to the category of metabolic process. Also, starvation test was conducted to study the affect of the light exposure on metabolic processes. Dim light causes flies to become more vulnerable to metabolic stress in wild type flies, while there was no difference in metabolic resistance pattern between circandian clock gene knock-out mutant flies and those exposed in dim light. These results imply that even a short-term dim light exposure affects gene expression and related metabolic pathways in *Drosophila melanogaster*. Also, this study may contribute to the understanding of metaboilic disorders by light pollution through elucididating the mechanisms of circadian disruption by light pollution.

603B

Metabolomics approach to understand the effect of genotype and diet on metabolic profile of Drosophila. Vishal Oza, Laura Reed. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Metabolic Syndrome (MetS) is a complex disease that involves a group of risk factors that increase the risk for heart disease and diabetes. The prevalence of MetS can be attributed to the westernized dietary habit and sedentary lifestyle. In this study, we used Drosophila to study the effect of genotype and different types of diet on the metabolic profile of the organism. Metabolite data was obtained from 16 genotypes representing two phenotypic groups for triglyceride storage on normal and high fat diets, using LC/MS and GC/MS platforms. We were able to detect 350 metabolites, 270 of which have definitive chemical IDs. The data was subjected to multivariate statistical analysis based on classification and regression methods. The metabolite profiles varied between two diets, indicating differences in the underlying biological pathways used by the organism based on diet and triglyceride storage levels.

604C

Dietary effects on the association between metabolite and gene expression using eigenvector metabolite analysis. Clare Scott[1], Ronglin Che[2], David Reif[3], Allison Montsinger-Reif[4], Laura Reed[1]. 1) Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) SAS, Raleigh-Durham, NC; 3) Genetics, NC State University, Raleigh, NC; 4) Bioinformatics Research Center, NC State University, Raleigh, NC.

Metabolic Syndrome (MetS) is a complex disease, which manifests symptoms including central obesity, insulin resistance, and elevated blood pressure. The occurrence of this disease, which leads to a greater risk of heart disease and type-2-diabetes, has been increasing. Interactions between genetic and environmental effects (Westernized diet and sedentary lifestyle) promote MetS, and these effects also influence metabolite expression. In this study, we quantified the metabolomes of 20 genotypes of *Drosophila melanogaster* that were reared on four different diets. We clustered the metabolites based on correlations and recovered chemically related groups of metabolites within the clusters. The linkages between correlated metabolite clusters, gene expression, and MetS phenotypes were characterized using the first principal component of each cluster. These analyses demonstrated that some clusters of metabolites are significantly correlated with groups of genes enriched for physiological processes that utilize these metabolites. Specific MetS phenotypes were also found to associate with specific metabolite clusters in a diet specific manner indicating context dependent mechanistic links between the MetS phenotypes and diet.

605A

Altered *dSERF* gene expression impacts protein homeostasis during aging and in neurodegenerative disease models. Swagata Ghosh, Sarah Milian, Alice Bevins, Douglas Harrison, Brian Rymond. Biology Dept, University of Kentucky, Lexington, KY.

Impaired protein homeostasis is known to play a role in several age-associated neurodegenerative diseases. *SERF1*, a phylogenetically conserved gene first identified as a putative genetic modifier of spinal muscular atrophy (SMA) was recently shown to promote amyloid protein aggregation in vitro and in vivo (Van Ham et.al, 2010; Falsone et.al, 2012). The natural function of *SERF1* is unknown. Here we investigate the impact of altered *dS*ERF1 gene expression on longevity and in established Parkinson's and SMA disease models. Using *dSERF* deletion and UAS-*dSERF*-cDNA transgenic expression lines we show that lifespan is diminished in the absence of *dSERF* and extended when this gene is ubiquitously mis-expressed. The *dSERF* deletion mutants show a correlative increase in the abundance of age-related polyubiquitinated protein aggregates in the thoracic muscles. In contrast with these natural polyubiquitinated aggregates, we find that the loss of *dSERF* expression decreases the abundance of α-synuclein protein in a fly model of Parkinson's disease. These results contrast with similar studies in *C. elegans* l, where *the loss of SERF/MOAG-4* activity was reported to reduce α-synuclein aggregate size but not α-synuclein protein abundance or *C. elegans* lifespan. Our α-synuclein results and related studies focusing on a fly Smn-limited SMA model suggest that *dSERF1* may act to modulate the efficiency of protein clearance.

606B

The effect of long-term selection on *Drosophila melanogaster* in a high-fat environment. Tanner L Hallman, Laura K. Reed. University of Alabama, Dept. of Biological Sciences, Box 870344, Tuscaloosa, AL 35487.

Through observation and previous research, it has been established that Drosophila melanogaster have a relatively poor survival rate on a high-fat diet. We are conducting a long-term selection experiment on 15 genetically unique populations in order to compare survival, adult and pupa weights, and time for each new generation to turn over using both a normal and high-fat diet. Each population was established from F1 hybrids between two genetically distinct founders derived from the Drosophila Synthetic Population Resource (DSPR). These hybrid genotypes have demonstrated substantial variation in survival reaction norms across the normal and high-fat diet in previous experiments. We anticipate that the environmental perturbation – the high-fat food – will expose cryptic genetic variation by decanalization, allowing selection to act despite the small effective population size. We hypothesize that populations derived from genotypes with the most dramatic reaction norms will also show the most rapid adaptation to the high-fat diet. .

607C

Population variation in phenotypic robustness to dietary perturbation. John C Henderson, Laura Reed. University of Alabama, Dept. of Biological Sciences, Box 870344, Tuscaloosa AL 35487.

The model of canalization proposes that populations attempt to maintain equilibrium in the face of stress by gene by genetically adaptive mechanisms. It has also been shown that populations differ genetically when geographically isolated. This study explores how geographically isolated populations differ in response to the stress of a perturbing diet, and to measure the levels of decanalization due to this stress. We tested ten isofemale lines from each of four populations recently gathered from the wild. The populations represent a broad geographic range of Drosophila melanogaster's distribution. To measure decanalization, we will analyze pupal weight variation within and between the populations for flies raised on either a normal or high fat diet. We found substantial variation between populations in their degree of phenotypic robustness to dietary perturbation. We hypothesize that the varied demographic and ecological history of the populations has influenced their ability to buffer the effects of a change in diet.

608A

The influence of mutant gene *Cam* on life span, moving activity and Ca^{2+} concentration in dystrophy mutants of *Drosophila melanogaster*. Nataliia Holub, Vasylyna Borutska, Khrystyna Dronska, Yaroslava Chernyk. Genetics & Biotechnology, National University, Lviv, Ukraine, Hrushevskogo Str., 4.

Duchenne muscular dystrophy is fatal X-linked myopathy characterized by muscular degeneration. Among different therapeutic strategies one is aimed using genes-modifiers of dystrophin-dystroglycan complex function. It is know that Ca^{2+} together with calmoduline regulate multiple pathways involved in skeletal muscle development. The aim of our work was to check the influence of mutant gene *Cam* on Ca^{2+} concentration in dystrophy mutants of *Drosophila melanogaster*. Strains *Oregon* (wild type), *DysDf//Tm6,Tb* (with deletion of dystrophy gene) and *Cam//CyO* (with loss of allele function) were used. First we have build survival curves and analyzed the indexes of median (MedLS) and maximum (MaxLS) life span in experimental strains. In *Oregon* flies indexes of MedLS were: S_{75} – 22 days, S_{50} – 29 days, S_{25} – 29 days; MaxLS arrived to 43 days. Dystrophy mutants characterized by decreased vitality and reduced indexes of MedLS: S_{75} – 8 days, S_{50} – 12 days, S_{25} – 22 days; MaxLS - 29 days. Increasing of MedLS and MaxLS comparing to dystrophy mutants observed in *Cam//+ DysDf//+* hybrids: S_{75} to 12 days, S_{50} to 19 days, S_{25} to 29 days; MaxLS to 36 days. In next experiment indexes of moving activity (IMA) were measured in 1-3, 4-6, 7-9 and 10-12-days imago. It was revealed that in hybrids IMA were in 1,6–2 times higher comparing to dystrophy mutants but in 1,5–1,9 times lower than IMA of *Oregon*. In examine strains concentration of Ca^{2+} and total protein level were researched on 1–3 and 10–12 days. It has been shown that Ca^{2+} concentration in 1–3 days flies *Oregon* was 0,31, in *DysDf//Tm6,Tb* – 0,22, in *Cam//CyO* – 0,38 and in *Cam//+ DysDf//+* hybrids – 0,27. In 10–12-days flies Ca^{2+} concentration increased up to 0,63, 0,38, 0,66 and 0,43 respectively. Reliable difference in total protein level in *Oregon, DysDf//Tm6,Tb, Cam//CyO* and hybrids flies of the same age (on 1–3 and 10–12 days) was not shown. Ca^{2+} concentration and total protein level were higher in females comparing to males.

609B

Myopathic lamin mutations cause reductive stress and activate the CncC (Nrf2)/Keap-1 pathway. Grant H. Young[1], George Dialynas[2], Om K. Shrestha[3], Jessica M. Ponce[4], Dylan A. Thiemann[1], Steven Moore[5], Liping Yu[1,6], Lori L. Wallrath[1]. 1) Department of Biochemistry, University of Iowa, Iowa City, IA 52242; 2) Stowers Institute for Medical Research, 1000 E. 50th St., Kansas City, MO 64110; 3) Department of Biochemistry, University of Wisconsin, Madison, WI 53706; 4) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA 52241; 5) Department of Pathology, University of Iowa, Iowa City, IA 52241; 6) NMR Facility, Carver College of Medicine, University of Iowa, Iowa City, IA 52241.

Mutations in the human *LMNA* gene cause muscular dystrophy by mechanisms that are not completely understood. The *LMNA* gene encodes A-type lamins, intermediate filaments that form a network underlying the inner nuclear membrane. To better understand pathomechanisms of mutant lamins, we performed structural and functional analyses on *LMNA* missense mutations identified in muscular dystrophy patients. These mutations cause changes in the tertiary structure of the A-type lamin Ig-fold domain. To identify the functional consequences of these structural perturbations, we made the corresponding mutations in Drosophila *Lamin C* and expressed the mutant lamins in larval body wall muscle. Affected muscles showed cytoplasmic aggregation of nuclear envelope proteins. Transcription profiling revealed upregulation of CncC (Nrf2) target genes. CncC is normally sequestered in the cytoplasm by

Keap-1. Under oxidative stress, Nrf2 dissociates from Keap-1, enters the nucleus and activates target genes. Unexpectedly, biochemical analyses revealed high levels of reducing agents, indicative of reductive stress. The abundance of cytoplasmic aggregates leads to elevated levels of the autophagy adaptor p62/SQSTM1, which competitively binds Keap-1, abrogating interactions with Nrf2 and allowing Nrf2 to enter the nucleus and activate gene expression. Elevated p62/SQSTM1 and nuclear enrichment of Nrf2 were identified in muscle biopsies from corresponding muscular dystrophy patients, validating the disease relevance of our Drosophila model. Our findings suggest new avenues of therapeutic intervention for lamin-associated muscular dystrophy that include regulation of protein folding and metabolism, as well as maintenance of redox homoeostasis.

610C

Insulin-like Signaling Genes *Chico* and *PGI* Significantly Modify Neurotoxicity in Parkinson's Disease. Rami Ajjuri, Janis O'Donnell. The University of Alabama, Tuscaloosa, AL.

Neurodegeneration is a naturally occurring process characterized by the gradual loss of neuron structure and function over time. As a result, aging remains the greatest factor contributing to the development of neurodegenerative disorders, such as Parkinson's disease (PD). While many genetic and environmental components have been linked to both familial and sporadic PD, the causes of over 85% of PD cases remain unknown, and are thought to result from a combination of both unidentified genetic factors and undetermined environmental triggers. In recent years, the role of the insulin/insulin-like growth factor signaling (IIS) pathway in influencing cellular longevity and health has become an immerging trend in neuroscience. Current evidence in yeast, worms, flies and mice strongly associates the IIS pathway in modulating aging, as well as in altering sensitivity to oxidative stress. Moreover, researchers are now investigating how age-related factors of the IIS pathway may directly and indirectly modify the progression of neurodegenerative diseases. Resulting from a biased genetic modifier screen, we identified two proteins- Insulin Receptor Substrate (IRS)/Chico and Glucose-6-phosphate isomerase (GPI)/PGI- involved in insulin signaling and glycolysis, respectively, that were able to alter PD pathogenesis. We report that mutations in the genes encoding these proteins resulted in significant modifications in dopamine neuron toxicity, dopamine regulation and nitric oxide-mediated neuroinflammation. Additionally, we observed that the treatment of both 2-DG, an inhibitor of glycolysis, and dietary glucose dramatically altered motor function in flies expressing human *alpha-synuclein*, a genetic hallmark in familial PD. Our collaborative studies in *C. elegans* and mouse primary neuron cultures further verify the conserved nature of these effects and provide strong evidence for the role of insulin signaling and glucose metabolism in modulating proteostasis and neurodegeneration.

611A

Creb Binding Protein and histone acetylation levels in a *Drosophila* model of MJD. Colin Beals-Reid, John Warrick. Biology, University of Richmond, Richmond, VA.

Ataxin 3 is the protein that is responsible for the autosomal dominant neurodegenerative condition Machado Joseph Disease (MJD). There is no known effective cure or therapy for this fatal disease. MJD is caused by an extended polyglutamine repeat stretch in the Ataxin 3 protein. Misfolded protein builds up over the life of cells, primarily neurons, that express Ataxin 3 and the disease eventually leads to death. We are investigating the molecular basis for MJD in a *Drosophila melanogaster* model. Our model expresses normal human Ataxin 3 and disease length Ataxin 3 in *Drosophila*. Previous work has shown hypoacetylation of histones in flies with MJD, contributing to the disease phenotype (Yi *et al*, 2013, PLoS One). Others showed that up-regulating CREB binding protein (dCBP or nejire) has phenotypic rescue effects and reverses hypoacetylation in fly model for polyglutamine diseases (Taylor *et al*. 2003, Genes and Development). However, we have found in our model that up-regulating dCBP has deleterious effects on degeneration while down regulation slows degeneration. We are now investigating the interactions between dCBP and histone acetylation in our model to understand the role of dCBP in our model. To measure histone acetylation, we isolate histones from the heads of flies and perform Western Blotting to visualize levels of histone H3 and H4 acetylation as compared to total histone H3. Preliminary results have shown that flies in our model do show hypoacetylation of histones when expressing disease length proteins. Further, down regulating dCBP knocks acetylation levels down in flies expressing the non-disease allele without additional loss of acetylation in flies expressing the disease causing allele. As well, treatment of flies with sodium valproate, a histone deacetylase inhibitor, does show restoration of histone acetylation levels in disease flies. We are currently examining the anatomy of flies treated with sodium valproate to see if they are rescued.

613C

The role of the glutamine-glutamate cycle in neuronal degeneration induced by the mutant human Huntington PolyQ protein. M.E. Pasini[1,3], M. Raneli[1], L. Vernizzi[1], N. Trong Tue[2], **P. Bellosta**[1,3]. 1) Dept of Biosciences, University of Milan, Milan, Italy; 2) Center for Gene-Protein Research Hanoi Medical University, Hanoi, Vietnam; 3) Ministero degli Affari Esteri e Cooperazione Internazionale Piazzale della Farnesina, 1 00135 Roma.

Huntington's disease (HD) is an inherited neurodegenerative disease caused by expansion of a CAG trinucleotide repeat in the first exon of the *Huntingtin* gene (*Htt*), which results in translation of a protein containing an enlarged CAG- encoded polyglutamine (polyQ) domain. HD is characterized by the progressive degeneration of neurons in the brain during aging. This results in chorea (involuntary movement), dementia, and death. The glutamate-glutamine cycle is fundamental in maintaining neuronal function and survival. When glutamate homeostasis is perturbed its levels in neurons increase, resulting in neuronal death. Glial cells also have an important function in maintaining this homeostasis, and glutamate removal from the synaptic cleft by glial cells is reduced in a mouse HD model suggesting that glia actively participate in the survival of neurons cells in HD. *But how does the glutamate-glutamine cycle contribute to*

neuronal death? And how does signaling in glial cells contribute to the amelioration of pathogenesis in HD? To answer these questions we are using a *Drosophila* model that has been successfully used to dissect the cellular and molecular events in polyQ-related diseases including HD. Our preliminary data show that manipulation of components of the glutamine-glutamate cycle rescues neuronal death and ameliorate the motility of animals expressing the HttQ93 in neurons using ELAV. Activation of TOR signaling inhibits autophagy that functions in neurons as a cleaning/survival mechanism. We would like to understand how components of the TOR pathway act in the regulation of the glutamate-glutamine cycle, and what controls autophagy in neuronal cells. *With this approach we hope to provide novel insights into the processes that cause neuronal degeneration not only in Huntington's diseases but also in other neuronal pathologies related to the aging of brain cells.* This work was sponsored by the Ministero degli Affari Esteri e Cooperazione Internazionale.

614A

Effects of nicotine on mitochondrial morphology and turnover in a *Drosophila park^{25}* mutant model of Parkinson's disease. Juliana M Cackovic, Gerald Call, Krista Pearman, Justin Smith, Lori Buhlman. Biomedical Sciences, Midwestern University, Glendale, AZ.

Parkinson's disease (PD) is characterized by a selective degeneration of dopaminergic neurons in the substantia nigra *pars compacta*. Mutations in the Parkin protein, an cytosolic ubiquitin ligase, are the most common cause of autosomal recessive juvenile parkinsonism[1]; Parkin facilitates fission of dysfunctional mitochondria preceding mitophagy [2]. Patients and with *parkin* mutations have increased mitochondrial connectivity and decreased electron transport chain (ETC) complex I activity in fibroblasts [3]. Defects in mitophagy may lead to decreased clearance of and eventual accumulation of dysfunctional, burdensome mitochondria, which could increase oxidative stress and cause cell death. Homozygous mutant *Drosophila* have decreased mitochondrial function that selectively affects dopaminergic neurons [4]. We have utilized *park^{25} Drosophila*[5] expressing mitochondrially tagged GFP in tyrosine hydroxylase (TH)- and choline acetyltransferase (Cha)- producing cells to determine the effect of Parkin loss of function on mitochondrial morphology and turnover in dopaminergic (DA) and cholinergic (ACh) neurons. Several studies have indicated that nicotine may protect against incidence and onset of PD by up regulating the release of dopamine in the striatum via modulation of nicotinic acetylcholine receptors (nAChR)[6], while other studies suggest that nicotine may be protective in an nAChR-independent manner [7,8]. Nicotine has been shown to increase lifespan and rescue deficits in olfaction and motility *park^{25} Drosophila*; however, the mechanism by which nicotine improves the phenotype is unknown [9]. Our final aims will address the mechanism by which nicotine protects against the *parkin* mutant phenotype by exploring the effects of nicotine on mitochondrial morphology and turnover in mutant *Drosophila* DA and ACh neurons. Our results will shed light on pathology of Parkin loss of function and provide evidence as to whether nicotine protects against the *parkin* mutant phenotype by affecting mitochondrial morphology and mitophagy in DA and/or ACh neurons.

615B

Study of APP protective pathway in *Drosophila*. Marlene Cassar, Jill Wentzell, Bonnie Bolkan, Katia Carmine-Simmen, Doris Kretzschmar. Oregon Institute of Occupational Health Sciences, OHSU, Portland, OR.

A key factor in Alzheimer's pathogenesis is the generation of the small Aß peptide, which is processed from Amyloid Precursor Proteins (APPs). However, APP processing also results in several other fragments, including soluble N-terminal ectodomains (sAPPs) and C-terminal intracellular domains (AICD). Despite the fact that APP is extensively studied, little is known about its normal function. Previous studies in our laboratory suggested that APPL (the fly ortholog of APP) signalling through its intracellular domains protective in a fly mutant that induces progressive neurodegeneration. Like in the human AICD, the Drosophila AICD contains several putative interaction domains, including a binding site for the G-protein Go-α. In *Drosophila*, Go-α proteins are widely expressed in the brain and are required for learning processes. We therefore hypothesized that Go-α is involved in the protective function of APPL previously observed in the AMPK/*loe* mutant. Using AMPK/*loe* pan-neuronal knockdowns as the genetic background, we found that overexpression of wild type or constitutively active forms of Go-α are protective against locomotion defects induced by *loe* inhibition whereas Go-α inhibition slightly decreased locomotion capabilities. Our preliminary results also suggest that this effect could be mediated by modulation of Transient Receptor Protein-Like (TrpL) calcium channels. We found that overexpression of TrpL in the *loe* knockdown background was also protective against progressive neurodegeneration observed in the mutant, whereas inhibition via RNA interference seemed to have little effect. Together, our results suggest that Go-α is part of the neuroprotective pathway initiated by APPL signalling and that Go-α modulates TrpL channels in order to mediate this protective effect. .

616C

The role of intracellular dopamine handling in selective degeneration of dopaminergic neurons in *Drosophila* Parkinson's disease models. Antonio Tito[1,3], **Shebna Cheema**[4], Zhen Xu[1], Yanning Rui[1], Zhihua Chen[1], Wen-Ting Li[5], Mian Jiang[4], Hugo Bellen[6,7], Sheng Zhang[1,2,3]. 1) The Brown Foundation Inst. of Molecular Med; 2) Dept of Neurobiology and Anatomy; 3) Human & Molecular Genetics and Neuroscience, University of Texas Graduate School of Biomedical Sciences The University of Texas Medical School, UTHEALTH; 4) University of Houston- Downtown Houston, TX, 77030; 5) Rice University; 6) Program in Developmental Biology; 7) Dept. of Molecular & Human Genetics and Neuroscience Baylor College of Med. Jan and Dan Duncan Neurological Research Inst.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the prominent loss of dopaminergic (DA) neurons in the Substantia Nigra, a region of the mesencephalon that plays an important role in motor control and goal-directed behaviors. Recent studies of two PD genes; *parkin* and *Pink1,* genes responsible for autosomal-recessive juvenile PD (AR-JP), suggest important role of mitochondria dysfunction in PD pathogenesis. Still, the molecular mechanisms underlying selective degeneration of DA neurons in PD are unknown. DA, the neurotransmitter synthesized by DA neurons, is highly unstable due to its unique chemical structure, prone to

oxidation in a basic environment with concomitant production of toxic reactive oxidative species (ROS). Accordingly, DA itself has long been suspected to play a role in the etiology of PD. In DA neurons, dopamine is uptaken into the cell and sequestered into synaptic vesicles (SVs) by dopamine active transporter (DAT) and vesicular monoamine transporter (VMAT), raising a possibility that their dysregulation might contribute to the disease development. To test the hypothesis, MiMIC-based eGFP-tagging and Gal4-trap lines were generated and studied for *in vivo* expression and subcellular localization of VMAT. We further characterized several UAS-VMAT transgenes and *vmat* mutant alleles for their gain- and loss-of-function phenotypes in whole animals and in DA neurons. Using these tools, we could genetically manipulate levels of cytoplasmic DA and different genetic background. We are currently determining the physiological effect of ex-vesicular DA on the survival of DA neurons in the brains of wildtype or *parkin* and *Pink1* mutant flies, and the resulting findings will be presented.

617A

Natural genetic modifiers of autosomal dominant retinitis pigmentosa. Clement Chow, Keegan Kelsey, Mariana Wolfner, Andrew Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Retinitis pigmentosa (RP) is characterized by progressive loss of vision due to degeneration of rods and cones in the retina. Autosomal dominant retinitis pigmentosa (ADRP) makes up ~40% of RP cases. Dominant mutations in the *rhodopsin* gene (*RHO*) comprise 25% of all ADRP cases. Dominant mutations in the *Drosophila melanogaster* ortholog of *RHO, Rh1* (or *ninE*), provide an important model for dissecting the pathophysiology of ADRP. The pathogenicity of the $Rh1^{G69D}$ mutation in *Drosophila* closely resembles that of many human mutations in *RHO*. $Rh1^{G69D}$ results in a misfolded protein that is retained in the endoplasmic reticulum (ER), and induces the ER stress response, leading to apoptotic cell death and retinal degeneration. Previous studies demonstrated that mutations in genes in the ER stress and apoptosis pathways can alter the phenotypic presentation of $Rh1^{G69D}$. However, these studies relied on loss-of-function (LOF) mutations. It is unlikely that severe LOF mutations contribute appreciably to variability in ADRP phenotypes; thus methods are needed to identify other types of modifiers. We took advantage of natural genetic variation in *Drosophila* to identify dominant modifiers of $Rh1^{G69D}$induced retinal degeneration. We crossed the $Rh1^{G69D}$ mutation into 173 *Drosophila* Genetic Reference Panel (DGRP) strains. To assess the effect of DGRP backgrounds on the phenotypic impact of$Rh1^{G69D}$, we measured eye size to quantify the extent of degeneration. Eye size varied by more than ten standard deviations. Given that such background modifiers caused over two-fold differences in eye size, we performed an association study to identify natural genetic polymorphisms that modified the $Rh1^{G69D}$ retinal degeneration. Novel candidates include genes which are involved in ER stress response, are known to modify the Rh1 protein, or have orthologs involved in other human retinal degenerative diseases. We used eye-specific RNAi and gene interaction studies to validate the role of the novel candidate genes in modifying the $Rh1^{G69D}$ phenotype. The candidate genes identified in this study may more accurately predict human modifiers of ADRP.

618B

Enhanced protein degradation protects against neurodegeneration in a *Drosophila* model of *c9orf72*-ALS. Kathleen M Cunningham[1], Ke Zhang[1], James Machamer[1], Thomas Lloyd[1,2]. 1) Department of Neurology, Johns Hopkins University, Baltimore , MD; 2) The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD.

Recently, a $(GGGGCC)_n$ hexanucleotide repeat expansion (HRE) in an intron of the *c9orf72* gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS), a degenerative disease of motor neurons. One of the major pathologies of c9-ALS is proteinaceous aggregates in the cytosol bound to p62. In a *Drosophila* model of c9-ALS expressing $(GGGGCC)_{30}$, we find p62 upregulation and p62-positive aggregates in motor neurons and impaired synaptic transmission at neuromuscular junctions. p62 plays a key role in autophagy by binding ubiquitinated proteins and delivering them to the autophagosome for degradation via the lysosome. p62 also reciprocally regulates protein degradation by the proteasome by blocking binding and delivery to the proteasome. We tested for the ability of genetic and pharmacologic manipulation of autophagy, the ubiquitin-proteasome pathway, and chaperones to rescue neurodegeneration caused by HRE expression. We find that although there are no defects in autophagic flux, the chaperone Hsp70 is severely downregulated in HRE-expressing flies. We propose that *c9orf72*-HRE expression causes dysregulation of protein folding and degradation leading to cytotoxic protein aggregations, which are rescued by clearance through genetic and pharmacological upregulation of chaperones, autophagy, and the ubiquitin-proteasome system. This study suggests that drugs targeting activation of protein degradation and folding pathways should be examined as therapies for the treatment of *c9orf72*-mediated ALS. .

619C

Engineered Hsp70 suppresses amyloid-β neurotoxicity in Drosophila by binding extracellular amyloid-β aggregates. Pedro Fernandez-Funez, Jonatan Sanchez-Garcia, Lorena de Mena, Yan Zhang, Diego E Rincon-Limas. Dept Neurology, Univ Florida, Gainesville, FL.

Alzheimer's disease (AD) is the most prevalent of a large group of related proteinopathies for which there is currently no cure. AD is characterized by the accumulation of the Aβ42 peptide in extracellular plaques and tau in neurofibrillary tangles. Both Aβ42 and tau are critical for inducing synaptic dysfucntion and neuronal death, although Aβ42 is proposed to by the intial trigger in AD. Here, we use*Drosophila* to explore a new strategy to block Aβ42 neurotoxicity through engineering of the Heat shock protein 70 (Hsp70), a chaperone with demonstrated neuroprotective activity against several intracellular amyloids. To target the protective activity of Hsp70 against extracellular Aβ42, we added a signal peptide to Hsp70. This engineered Hsp70 (secHsp70) is efficiently secreted in S2 cells and in transgenic flies. When combined with Aβ42, secHsp70 suppresses Aβ42 neurotoxicity in adult eyes, reduces cell death in eye discs and brain neurons, protects the structural integrity of adult neurons, alleviates locomotor dysfunction, and extends lifespan. We also

demonstrate that secHsp70 directly binds Aβ42 and this interaction blocks Aβ42 neurotoxicity without reducing amyloid-β levels or aggregation, suggesting that the protective activity of secHsp70 is mediated by "masking" neurotoxic Aβ42 epitopes. We have recently determined that the ATPase domain is not required for the protective activity of secHsp70, which is consistent with the lack of ATP and co-factors in the extracellular space. This result suggests that secHsp70 does not protect through its well-documented refolding activity and instead acts as a holdase. We are currently testing the effect of mutations in the substrate-binding domain of secHsp70, which we expect will block its protective activity. Combined with other approaches, this novel strategy may contribute to reduce the burden of AD and other extracellular proteinopathies.

620A

Role of a soy protein Lunasin in Aβ42 mediated neurodegeneration in Alzheimer's Disease. Angela Giaquinto[1], Michael Moran[1], Ankita Sarkar[1], Gillian Jones[2], Ajay Srivastava[2], Maduri Kango-Singh[1,3,4], Amit Singh[1,3,4]. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 2) Department of Biology and Biotechnology Center, Western Kentucky University, 1906 College Heights Blvd, Bowling Green, KY; 3) Premedical Program, University of Dayton,300 College Park Drive, Dayton,OH; 4) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's Disease (AD) is a neurodegenerative disease caused by a number of factors. One of the leading factors behind the onset of AD is the accumulation of amyloid plaques in the brains of affected individuals. These plaques are formed with amyloid precursor protein (APP) is processed incorrectly and cleaved to be 42 amino acids long (Aβ42) instead of 40 (Aβ40). These two extra amino acids cause the protein to become hydrophobic in nature and form plaques which aggregate around neurons in the brain. This aggregation induces oxidative stress on the neurons which then leads to cell death. Due to the conserved genetic properties of the*Drosophila melanogaster*, fruit fly, visual system with that of humans we have developed a *Drosophila* eye model. In this model the Aβ42 protein is misexpressed in the developing photoreceptors of the fly eye which results in extensive cell death of the photoreceptor neurons and produces a highly reduced eye field in the adult fly. Our aim is to understand the function of a soy protein called Lunasin in Alzheimer's disease. It has been shown that Lunasin acts as an anti-inflammatory within the somatic cells. Inflammation is also one of the characteristic of AD. Therefore, we investigated the effects of Lunasin on Aβ42 accumulation mediated neurodegeneration. Here we present the findings of our studies.

621B

Evaluating the potential therapeutic role of ACE inhibitors for Alzheimer's Disease in Drosophila. Sarah M Gomes[1,2], Gabrielle L Boulianne[1,2]. 1) The Hospital for Sick Children, Toronto, Canada; 2) University of Toronto, Toronto, Canada.

Presenilins (PS) are highly conserved proteins that were identified as causative factors in familial Alzheimer's Disease (AD) but have since been shown to play a critical role in development by regulating the Notch signaling pathway. PS function as the catalytic core of the γ-secretase complex, which cleaves transmembrane proteins such as Notch and APP. In the case of Notch, PS cleavage results in the release of an intracellular domain, which can translocate to the nucleus and activate downstream target genes. In the case of APP, PS cleavage results in the release of secreted amyloid (Aβ peptides), which have been widely suggested as a primary cause for AD-related neurodegeneration. To further characterize the role of PS in development and AD, we previously performed a genetic screen for modifiers and identified Acer and Ance-5, two orthologs of human Angiotensin Converting Enzyme (ACE), a metalloprotease of the renin-angiotensin system. Genetic polymorphisms in human ACE have been associated with sporadic AD and have been shown to associate with Apo-E, the best characterized risk factor for late-onset AD. Additionally, ACE inhibitors have been shown to delay the onset of cognitive impairment and neurodegeneration in AD mouse models. Moreover, recent studies have reported a significant reduction in the incidence of AD among subjects taking ACE inhibitors. At present, it is unclear why ACE inhibitors are beneficial in AD. However, several studies have shown that the ability of ACE inhibitors to reduce the incidence of AD is independent of its ability to lower blood pressure. Here, we further explore the genetic interaction between PS and ACE orthologs in Drosophila. We further examine how loss of Acer and Ance affects AD related phenotypes in Drosophila models using genetic and pharmacological methods (e.g. ACE inhibitors captopril and losartan). Finally, we examined the effect of ACE inhibition on Notch signaling. If inhibition of ACE has no detrimental effect on the Notch pathway and still modulates AD related phenotypes, it could provide an important therapeutic target for AD.

622C

Neuroinflammation and Hypoxia in a Neurodegenerative Disease Model. Marleshia Hall[1], J. Gavin Daigle[1,2], Rami Ajjuri[1], O'Donnell Janis[1]. 1) Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Department of Human Genetics, LSU School of Medicine, New Orleans, LA.

Neuroinflammation and hypoxia activated as a result of neurodegenerative disease or environmental insult have been widely studied. However, simple genetic models for these neuronal responses, which consequently result in disease progression, have been lacking. The herbicide paraquat induces selective dopamine neuron loss in Drosophila. We assayed whether paraquat induces neuroinflammation subsequently resulting in neuron loss and truncated lifespan. We report that paraquat induces neuroinflammation and an integrated hypoxia response whereby key regulators of both processes are activated. We also show that an inhibitor of NOS rescues truncated survival and dopamine neuron loss.

623A

Direct regulation of a conserved axon regeneration pathway by Protein Kinase A. Yan Hao, Catherine Collins. MCDB, University of Michigan, Ann Arbor, MI.

In order to inhibit the loss of axons and to promote their repair in cases of injury and disease, we need to understand the molecular pathways that promote both degenerative and regenerative responses to axonal damage. Towards these goals, our lab has implemented a molecular-genetic and live imaging approach that capitalizes on the rapid and powerful genetics of Drosophila as a model organism. Our work in this system has delineated an 'axonal injury response' pathway, whose mechanism is highly conserved from invertebrates to vertebrates. A central regulator of this pathway is an axonal mitogen activated protein kinase kinase kinase kinase (MAPKKK), named Wallenda (Wnd) in Drosophila and DLK in mammals, whose activation in response to axonal damage is essential for the ability of injured neurons in the peripheral nervous system (PNS) to regenerate. We have also found that Wnd activation promotes a protective response that inhibits axonal degeneration of damaged axons. Our current focus is to understand how this kinase detects axonal damage. Our recent findings have implicated Protein Kinase A (PKA) as an important upstream regulator of Wnd/DLK in axonal damage signaling. cAMP and PKA are long known for their roles in stimulating axonal regeneration in a wide range of cell types and organisms. In Drosophila motoneuron axons, PKA activation induces an additional protection against axonal degeneration, and this, as well as its pro-regenerative effects, requires Wnd function. Importantly, we have found that PKA activation directly modulates Wnd/DLK protein at a post-translational level in Drosophila motoneuron axons as well as in mammalian (HEK293) cells, and that PKA function is required for the acute increase in Wnd protein levels and downstream signaling after axonal injury. We will present our current view of the mechanism of axonal injury signaling, with PKA and Wnd/DLK functioning in a single highly conserved pathway.

624B

A high throughput screen for chemical modifiers of a *Drosophila* model for Niemann-Pick type C. Tom Hartl, Ethan Perlstein. Perlstein Lab PBC, San Francisco, CA.

Perlstein Lab PBC is a San Francisco based startup using model organisms to find small molecule therapies for patients with rare genetic diseases. The first disease we are targeting is Niemann-Pick type C, an autosomal recessive disorder caused by mutations in the *npc1* gene. *Drosophila* with *npc1* mutations are 20-hydroxyecdysone deficient and arrest at the first instar larval stage. Here I will describe our high throughput chemical modifier screen to identify compounds that can reverse the larval first instar arrest of flies carrying *npc1* mutations.

625C

Tau-spliceosome interactions in *Drosophila* models of Alzheimer's disease. Yi-Chen Hsieh[1], Martin P. Powers[1], Janson White[1], Joshua M. Shulman[1,2]. 1) Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Houston, TX.

At autopsy, Alzheimer's disease (AD) and related tauopathies are defined by the presence of insoluble, cytoplasmic aggregates comprised of the microtubule-associated protein tau, termed neurofibrillary tangles. Our collaborators recently revealed that U1-70K and several other snRNP spliceosome components co-aggregate with Tau in the insoluble proteome of AD brains. Independently, in a screen of conserved candidate genes from AD-associated human genomic loci, we discovered that *SmB*, the fly ortholog of human *SNRPN*, robustly interacts with the neurotoxicity of *human Tau* (*hTau*) in transgenic animals. Specifically, we find that RNAi-mediated *SmB* knockdown enhances the hTau-induced rough eye, and reciprocally *SmB* overexpression suppresses hTau retinal toxicity. The *SmB*-hTau interaction was further confirmed using a newly developed assay for hTau-mediated functional degeneration based on electroretinogram (ERG) responses in aged flies. Interestingly, we also find that knockdown of fly*U1-70K* is sufficient to cause progressive ERG deficits independent of Tau, suggestive of a downstream role. We hypothesize that sequestration of SmB, U1-70K, and perhaps other spliceosomal components in cytoplasmic tau aggregates may lead to global disruptions in the neuronal transcriptome, and ultimately neurodegeneration. We are currently testing this model using complementary conditional knockdown strategies to determine the consequences of SmB depletion in the adult central nervous system. We are also directly examining whether hTau can trigger the mislocalization and cytoplasmic co-aggregation of spliceosomal components in *Drosophila* neurons, as suggested by studies of human AD postmortem brains. In sum, our results begin to reveal a novel mechanism for Tau-induced neurodegeneration mediated by disruptions of the RNA splicing machinery, possibly leading to deleterious changes in global gene expression and/or splicing. .

626A

***Drosophila* eye model to understand role of signaling pathways in Aβ42 mediated neurodegeneration. Madison Irwin**[1], Meghana Tare[1,4], Michael Moran[1], Madhuri Kango-Singh[1,2,3], Amit Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH; 4) Department of Cancer Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA.

Alzheimer's Disease (AD), the 5th leading cause of death in elders, is a progressive neurodegenerative disorder without a cure that affects more than five million Americans. It is characterized by accumulation of Aβ42 peptides, which are toxic to neurons and lead to cell death. Earlier, we have shown that a highly conserved signaling pathway, the c-Jun amino-terminal (NH_2) kinase pathway (JNK), is involved in Aβ42 mediated neurodegeneration. Another highly conserved signaling pathway, the Hippo growth regulation pathway, is also thought to be involved in Aβ42 cell mediated neurodgeneration and may interact with JNK signaling. We have employed a Drosophila eye model for AD where human Aβ42 is misexpressed in the differentiating *Drosophila* retina to discern the role of the Hippo signaling pathway in Aβ42 mediated cell death. Using our transgenic fly eye model, which exhibits progressive loss of retinal

neurons as seen in Alzheimer's disease in humans, we found that the Aβ42 neurodegenerative phenotype can be rescued and enhanced by modulating Hippo signaling. Furthermore, the two pathways modulate each other. These studies aim to understand the genetic underpinnings of AD.

627B

Aberrant immune activation in the *Drosophila Adar* mutant neurodegeneration. Liam Keegan[1], Simona Paro[2], Leeanne McGurk[2], Xianghua Li[2], Niamh Mannion[2], Marion Hogg[2], Robert Young[2], Ian Adams[2], Rui Zhang[3], Jin-Billy Li[3], Giusy Pemmetta[4], Mary O,Connell[1]. 1) Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5, 625 000 Brno, Czech Republic; 2) MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine at the University of Edinburgh, Crewe Road, Edinburgh EH4 2XU, UK; 3) Department of Genetics, Stanford University, 300 Pasteur Dr., Stanford, CA 94305, USA; 4) 3Centre for Integrative Physiology, Euan MacDonald Centre for Motor Neurone Disease Research, Hugh Robson Building, University of Edinburgh, George Square, Edinburgh, EH8 9XD.

ADAR RNA editing enzymes deaminate adenosine bases to inosines in dsRNA. Vertebrate ADAR1 controls innate immune responses to cellular dsRNA and mutations in Adar1 cause Aicardi-Goutieres Syndrome, a childhood encephalopathy with interferon expression and symptoms resembling those caused by congenital virus infection. *Drosophila* has a single ADAR2-type enzyme that catalyses hundreds of site-specific editing events in CNS transcripts. *Adar* mutant flies show reduced viability, uncoordinated locomotion and age-dependent neurodegeneration, consistent with loss of recoding in ion channel transcripts. However, we demonstrate that aberrant induction of antimicrobial peptide (AMP), transcripts occurs in the *Drosophila Adar* mutant and that NF-κB *Rel* mutation rescues *Adar* mutant phenotypes. Surprisingly, a genetic screen for deficiencies rescuing the reduced viability of *Adar* mutant flies reveals that reduced *Tor* gene dosage rescues by increasing canonical autophagy. In *Adar1*mutant mice aberrant immune activation involves the innate immune sensor RIG-I Receptor (RLR) RNA helicases. *Drosophila* innate immune responses do not involve RLR signalling but may instead rely on unknown sensors, and immune-driven autophagy and AMP expression. Suppresion of neurodegeneration by preventing immune induction in Drosophila models is a major finding with implications for human neurodegenerative and psychiatric diseases.

628C

Role of Dpp signaling pathway in promoting survival of retinal neurons in Aβ42 mediated neurodegeneration. James Kirwan[1,2], Ankita Sarkar[2], Madhuri Kango-Singh[2,3,4], Amit Singh[2,3,4]. 1) Drexel University, School of Biomedical Engineering, Science, and Health Systems, 3141 Chestnut Street, Philadelphia, PA; 2) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Premedical Program, University of Dayton,300 College Park Drive, Dayton, OH; 4) Center for TissueRegeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's disease is a progressive neurodegenerative disorder with no known cure to date. One cause of Alzheimer's neuropathy is the generation of Amyloid-beta-42 (Aβ42) aggregates that trigger cell death by unknown mechanisms. Using a transgenic *Drosophila* eye model misexpressing human Aβ42, we observed the AD-like neuropathy. In a forward genetic screen we have identified Decapentaplegic (Dpp), a morphogen, as one of the genetic modifiers of Aβ42 mediated neurodegeneration. Dpp acts as the ligand for the *dpp* pathway, which exhibits suppression of retinal neuron's cell death. The Dpp signaling pathway involves several key components. We examined the Dpp signaling pathway and its members in modifying Aβ42 mediated neuropathy. The results from our studies will be presented.

629A

Characterization of the molecular mechanisms underlying *TARDBP* mutation toxicity in *drosophila*. Magalie Lecourtois, Laetitia Miguel, Tracey Avequin, Thierry Frebourg, Dominique Campion. Inserm U1079, Normandie University, IRIB, Rouen, France.

Physiologically, TAR DNA-binding protein-43 (TDP-43) is a protein involved in RNA processing and is primarily located in the nucleus, but continuously shuttles to and from the cytoplasm. In addition to transcription, pre-mRNA splicing, transport to and rapid local RNA translation at the synapse, and decay, TDP-43 proteins may also be involved in microRNA biogenesis. Recently, TDP-43 has been identified as a major constituent of nuclear and/or cytoplasmic ubiquitin-positive inclusions in patients with amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD). Pathological proteins are abnormally hyperphosphorylated and partially cleaved, generating C-terminal fragments. Dominant mutations in the TARDBP/TDP-43 gene are causative for familial ALS. Although the presence of TARDBP mutations is a clear indicator that dysfunction of TDP-43 is directly linked to neurodegeneration, the functional consequences of TARDBP mutations are still unresolved. Whether mutations exert their effects through gain or loss of function remains unclear. In order to address these aspects in vivo, we have developed new Drosophila transgenic lines expressing similar mRNA levels of wild-type or ALS-linked TDP-43 mutants, thanks to genomic targeted transgene insertion. Biochemical and phenotypic characterization of these flies are on-going and will be presented.

630B

The calcineurin inhibitor, sarah, exasperates Aβ42 phenotypes in a *Drosophila* model of Alzheimer's Disease. Soojin Lee, Se Min Bang, Yoon Ki Hong, Jang Ho Lee, Haemin Jeong, Seung Hwan Park, Kyoung Sang Cho. Biological sciences, Konkuk university, Seoul, Seoul, South Korea.

The Down Syndrome Critical Region 1 (DSCR1) protein, an inhibitor of Ca^{2+}-dependent phosphatase calcineurin, has been shown to be elevated in the brains of Down syndrome (DS) or Alzheimer's disease (AD) patients. Because DSCR1 has been implicated in the

neuropathology of DS, an increased level of DSCR1 is believed to be deleterious to neuronal health. On the other hand, because hyper-activated calcineurin has been implicated in neuronal damage, calcineurin inhibitors such as DSCR1 are expected to exert beneficial effects against AD neuropathology. However, the role of DSCR1 in the pathogenesis of AD is still unclear. Here, we investigated the role of *sarah* (*sra*)/nebula, a *Drosophila DSCR1* ortholog, in the amyloid-β42 (Aβ42)-induced neurological phenotypes in *Drosophila*. We detected transcriptional activities of the *sra* promoter in the mushroom body of the fly brain, which is the *Drosophila* counterpart of the human hippocampus. Moreover, similar to human AD patients, *Aβ42*-expressing flies showed an increased level of the *sra* transcription in the head, demonstrating that the expression pattern of DSCR1 with regard to AD pathogenesis is conserved in *Drosophila*. Interestingly, overexpression of the *sra* using the *UAS-GAL4* system exacerbated the rough eye phenotype, and decreased the survival rates of the *Aβ42*-expressing flies without modulating the *Aβ42* expression. Similarly, treatment with chemical inhibitors of calcineurin such as FK506 and cyclosporine A, or knockdown of *calcineurin* expression by RNAi, worsened the rough eye phenotype induced by *Aβ42*. Furthermore, the *sra*-overexpressing flies displayed significantly decreased mitochondrial DNA content, as well as increased susceptibility to oxidative stress compared to the control flies. Taken together, our results demonstrating that overexpression of *sra* has an augmenting role on Aβ42 cytotoxicity in *Drosophila* suggest that the *DSCR1* up-regulation or *calcineurin* down-regulation in the brains of DS or AD patients may accelerate neuropathogenesis of AD in humans as well.

631C

***Bcl-2* homologues, *Buffy* and *debcl*, influence Drosophila models of Parkinson Disease. P Githure M'Angale**, Brian E. Staveley. Biology Department, Memorial University Of NFLD, St. John's, NL, Canada.

In contrast to the complexity found in mammals, the *Bcl-2* family genes found in Drosophila consist of the pro-cell survival *Buffy* and the anti-cell survival *debcl*. We used *ddc-GAL4* to overexpress and inhibit the two *Bcl-2* homologues in the dopaminergic neurons of Drosophila. The overexpression of *debcl* and the inhibition of *Buffy* in the dopamine producing neurons resulted in flies with reduced climbing ability over time, similar to the phenotype seen in *α-synuclein*-induced Drosophila model of Parkinson Disease (PD), to generate novel models of neurodegenerative disease. In the *α-synuclein*-induced Drosophila model of PD, we altered the expression of *Buffy* and *debcl* in the dopamine producing neurons and analyzed longevity and climbing ability of these flies. When these two genes were overexpressed in the dopamine producing neurons, *debcl* enhanced the *α-synuclein*-induced loss of locomotion over time while *Buffy* acted to rescue this phenotype. In an analogous manner, when overexpressed in the developing eye using *GMR-GAL4*, we found that *debcl* led to significant deterioration of the ommatidial array: this can be partially rescued by co-expressing *Buffy*. When expressed using *GMR-GAL4*, *α-synuclein* induces disruption of the ommatidial array and when the *Bcl-2* family genes are overexpressed along with *α-synuclein*, *Buffy* suppresses and *debcl* enhances the severity of the *α-synuclein*-induced developmental defects of the eye. Taken together, these experiments suggest a potentially protective role for *Buffy* and a potentially detrimental one for *debcl* in *α-synuclein*-induced protein toxicity and possibly in Drosophila models of Parkinson disease. The mechanism of this protective role may be through the safeguarding of the mitochondria by pro-cell survival Bcl-2 proteins. (Funded by a MUN School of Graduate Studies Fellowship to PGM and by NSERC Discovery Grants to BES.).

632A

Quantitative analysis of climbing deficits and nicotine effects in homozygous *parkin* mutant flies. Brady Mannett[1], John M Grose[2], Krista Pearman[3], Lori M Buhlman[1], Gerald B Call[3]. 1) Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, AZ; 2) Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 3) Dept of Pharmacology, AZCOM, Midwestern University, Glendale, AZ.

Parkinson's disease (PD) is a neurodegenerative disorder in which the dopaminergic neurons from the *substantia nigra pars compacta* undergo degeneration that leads to motor and olfaction deficits in PD patients. A *Drosophila melanogaster* PD model with a mutant *parkin* gene has shown remarkable similarities to PD, including motor deficiencies, olfaction loss, dopaminergic neuron loss and a reduced lifespan (Chambers et al. 2013, Whitworth et al. 2005). Nicotine treatment has been found to improve motor deficits in flight and climbing, and has even been found to rescue olfaction loss in heterozygous *parkin*-deficient flies ($park^{25}$/+) (Chambers et al. 2013). Here we study nicotine treatment in homozygous *parkin*-deficient *Drosophila melanogaster* model ($park^{25}/park^{25}$) by measuring climbing in a newly developed assay utilizing a multibeam activity monitor to more quantitatively assess climbing deficiencies in these flies. When measuring climbing behaviors over a 20 minute period, 10- and 20-day-old $park^{25}$ mutants attempt to climb the same number of times as control (w^{1118}) flies. However, the $park^{25}$ flies have reduced total height climbed, a reduced number of climbs that reach the top of the climbing vial (successful climbs), a reduced average height climbed and a reduced velocity of climbing. When given nicotine (3 mg/ml or 4.5 mg/ml) in their food from day 0 post-eclosion, some climbing parameters show improvement, or even rescue to control levels; however, some interesting differences exist. For instance, there is a differential response for the percent of successful climbs with nicotine treatment: day 10 flies show no improvement with nicotine, but day 20 flies show rescue to control levels. More analysis of climbing defects, along with flight and olfaction in $park^{25}$ homozygotes and the effects of nicotine will be presented at the conference.

633B

Examining *spargel*, the homologue of *PGC-1α*, in *Drosophila* models of Parkinson Disease. Eric Merzetti, Brian Staveley. Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Parkinson disease (PD), a common and progressive neurological disease, features the clinical symptoms of resting tremor, bradykinesia, rigidity and postural instability. These arise from decreases in dopamine available in the striatum of the brain when

neurons responsible for the processing and production of dopamine become dysfunctional or die. Understanding the cause of this breakdown may lead to new and effective models and, eventually, novel strategies for PD prevention. Mitochondrial upkeep and repair involves a number of genes linked to neuronal dysfunction including *parkin*, *Pink1* and the peroxisome proliferation activated co-receptor gamma (PCG) family of genes. In mammals, *PGC-1α* has been characterised as a modulator of mitochondrial biogenesis, energy metabolism, insulin signalling and has been linked to the onset of PD. The study of *PGC-1α* in mammalian cells is made difficult by the functional redundancy of the other PGC family members, *PGC-1β* and *PRC* (PGC-1-related-cofactor). The only PGC family member present in *Drosophila* is the *PGC-1α* homologue *spargel (srl)* which codes for a 1,088 amino acid protein that exhibits 68% homology in the COOH-terminal RNA-binding motif, shares an arginine-serine rich domain, an acidic NH_2 terminal domain and a leucine-rich domain with the mammalian *PGC-1α*. We investigate *srl* as a potential key regulator of disease model progression. Using *Gal4* mediated tissue specific expression, we have determined that strong RNAi mediated inhibition of *srl* expression in the dopaminergic neurons alters lifespan, and locomotor ability in *Drosophila melanogaster*. Furthermore, *srl* expression seems to be tightly regulated as elevated expression also leads to altered longevity, locomotor ability and development. This model may be used to identify and characterize the relationship of other genes involved in mitochondrial synthesis, helping to further our understanding of mitochondrial links to neurodegenerative disease. Funded by School of Graduate Studies Fellowships to EMM and Parkinson Society Canada (Regional Partnership) and NSERC Discovery Grants to BES.

634C

Targeting the interaction of Ataxin-3 and Rad23 for therapeutic purposes in the neurodegenerative disease Spinocerebellar Ataxia Type 3. Joanna R Miller, Jessica Blount, Wei-Ling Tsou, Sokol Todi. PHARMACOLOGY, WAYNE STATE UNIVERSITY, DETROIT, MI.

Spinocerebellar Ataxia Type 3 (SCA3), which is also known as Machado-Joseph Disease, is a dominantly inherited neurodegenerative disorder characterized by a loss of muscle coordination, for which currently only symptomatic treatments exist. SCA3 is a CAG/polyglutamine disorder, with patients possessing expanded CAG nucleotide repeats in the *ATXN3* gene, which results in an abnormally long polyglutamine tract and misfolding and aggregation of the ataxin-3 protein. Since reducing levels of disease proteins improves pathology in animal models of polyglutamine diseases, including in SCA3, our lab investigated how ataxin-3 is degraded. We recently demonstrated that ataxin-3 does not require ubiquitination to be degraded. Rather, its turnover is regulated by ubiquitin-binding site 2 (UbS2) on its N terminus. Ataxin-3 is stabilized by its interaction with the proteasome-associated proteins Rad23A/B through UbS2. Mutating UbS2 to disrupt the interaction between ataxin-3 and Rad23A/B decreases ataxin-3 protein levels in cultured mammalian cells. These findings led us to conclude that UbS2 could be a potential target through which to enhance ataxin-3 degradation for SCA3 therapy. To test this possibility, we have generated new transgenic *Drosophila* lines that express either pathogenic ataxin-3 with all of its domains intact or pathogenic ataxin-3 with UbS2 mutated. We are currently characterizing these flies and I will be presenting results from experiments that test whether inhibiting the interaction of ataxin-3 with Rad23, which leads to lower ataxin-3 protein levels, also diminishes the pathogenic propensity of this disease protein in *Drosophila*.

635A

Understanding the Molecular Pathology of Spinal and Bulbar Muscular Atrophy by Identifying Genetic Interactors of an AR-Humanized Fly. Shaza Mokhtar[1,2*], Mark Trifiro[1,2,3], Miltiadis Paliouras[1,2,3]. 1) Human Genetics, McGill University, MONTREAL, QC, Canada; 2) Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; 3) Department of Medicine, McGill University, Montreal, QC, Canada.

Background**:** SBMA is an X-linked recessive neurodegenerative disease, that is a result of a polyglutamine (polyQ) expansion in the androgen receptor (AR). The toxic gain-of-function that is characteristic of SBMA resulting in a slow progressive muscle weakness, fasciculations of bulbar, muscle cramping. The AR is involved in a myriad of cellular functions that have a role in the development and growth of the male reproductive and nonreproductive systems, with functionality extending beyond the role as a transcription factor to include the novel properties of RNA splicing, proteasomal interaction, DNA methylation and affecting RNA translation at polyribosomes. The complexity of the AR-interacting protein and RNA could involve different pathways that function in the AR biological output. Drosophila genetic system uses for a high-throughput screen of large number of gene and genetic interactors that may decrease or increase the polyQ-AR/androgen-dependent phenotype.

Aims of the Study: We will study polyQ-AR protein and RNA initiating disease phenotype by using AR-humanized Drosophila disease mode, by expressing the polyQ-AR in the nervous system of the developing fly embryo. candidate genetic interactors of PolyQ-AR flies will be crossed with RNAi and homologous fly genes encoding AR candidate protein interactors.

Results**:** A proteomics study was used to establish protein composition of wild-type-AR (WT-AR) complexes vs. polyQ-expanded-AR (polyQ-AR) complexes. Date shows that AR-interacting partners change in the AR-interacting between polyQ-AR and the WT. There are differences in the ability of polyQ-AR to interact with protein that presented by RNA binding proteins.

Conclusion: Studying the role of AR in RNA-splicing and RNA-binding is a novel discovery. Using a Drosophila as a genetic interaction screen, will help us to understand the mechanism that is involved in SBMA disease.

636B

Roles of reticulons and REEPs in organisation of axonal endoplasmic reticlum in *Drosophila*. Belgin Yalcin, Niamh C O'Sullivan, Martin Stofanko, Zi Han Kang, Annika Hartwich, Matthew Thomas, **Cahir J O'Kane**. Department of Genetics, University of Cambridge, Cambridge, United Kingdom.

Failures of maintenance of long axons are seen in the Hereditary Spastic Paraplegias (HSPs), characterised by lower limb weakness,

and degeneration of longer upper motor axons. To date over 50 causative Spastic Paraplegia Genes (SPGs) have been identified. Several encode endoplasmic reticulum membrane proteins, with intramembrane hairpin structures that curve and model ER membrane. Two of these protein families, reticulons and REEPs, are together required for formation of most tubular ER in yeast. To test whether they are also required for formation or maintenance of axonal ER, we have developed suitable markers in *Drosophila*, and analyzed mutants that lack members of these protein families. *Drosophila* has two reticulon genes, one of which, *Rtnl1*, is widely expressed. YFP-tagged Rtnl1 localises strongly in axons and presynaptic terminals, in contrast to most conventional ER markers. *Rtnl1*knockdown reduces levels of smooth ER marker in longer distal motor axons, but not in shorter or proximal ones – analogous to HSP. This effect is also seen in labeled single axons, with no loss of ER continuity. *Drosophila* has six REEP genes, two of which are widely and highly expressed. ReepA (CG42678) is orthologous to mammalian REEPs 1 to 4, and ReepB (CG8331) to mammalian REEP5 and REEP6. We can detect ReepB::GFP but not ReepA::GFP in axons and presynaptic terminals. When both ReepA and ReepB are removed, ER organisation is altered in epidermal cells but appears normal in axons. However, triple mutants that remove Rtnl1, ReepA and ReepB show major disruption of ER in motor axons.

We have therefore established *Drosophila* motor axons as a system for assaying axonal ER organisation, and the roles of spastic paraplegia genes in this process. This will allow additional and newly identified HSP genes to be tested for such roles, either singly or in combination with others.

637C

Activation of the wallenda/DLK pathway triggers adult photoreceptor degeneration. Kirk Mecklenburg[2], Stephanie Freed[1], Forrest Weghorst[1], **Joseph O'Tousa**[1]. 1) Biology Dept., Indiana University at South Bend, South Bend, IN; 2) Dept. of Biological Sciences University of Notre Dame, Notre Dame, IN.

Wallenda (WND) is the *Drosophila* member of a conserved family of dual leucine kinases (DLK) playing a pivotal role in specifying a neuron's response to axon damage. WND activity is associated with both regenerative and degenerative effects in different cell types and can also trigger opposite effects within an individual neuron's dendritic field and axonal projection. The signaling pathway involves WND acting through Hemipterous (Hep) and Kayak/FosB, but the ultimate effectors driving the cellular responses are not known. We evaluated the role of WND overexpression in *Drosophila* photoreceptors by expressing UAS-*wnd* under control of the rhodopsin Rh1-GAL4 driver. In light-reared flies, Rh1-driven WND expression causes loss of the photoreceptors' rhabdomeres by eclosion, whereas this process is delayed in dark-reared animals. As rhabdomeres degenerate, rhodopsin is lost, and soluble rhabdomeric proteins are delocalized into the photoreceptor cell body. In contrast to these deficits seen in the photoreceptor cell body, the photoreceptor axons and end bulbs remain largely intact. RNAi knockdown of *hep* and *kayak* partially suppresses the WND-induced effects, indicating that these pathway components are expressed in photoreceptors and, importantly, that the photoreceptor cell's response to WND activity is mediated through the same canonical signaling pathway active in other neurons. Also in support of this view is the observation that Rh1-GAL4 driven expression of UAS-*hep* has similar effects as UAS-*wnd* on photoreceptor structure. Both the experimental accessibility and tools available make the *Drosophila* photoreceptor an excellent experimental system to identify the downstream cellular events that result from the WND/DLK signaling pathway.

638A

Co-Expression of human APP and BACE impairs short term memory as measured by the Aversive Phototaxis Suppression Assay in a *Drosophila* model of Alzheimer's Disease. Ashka Patel, Paddock Brie. Biology Dept, Arcadia University, Glenside,PA.

Alzheimer's Disease (AD) is a prevalent neurodegenerative disease characterized by memory loss and the accumulation of amyloid plaques and neurofibrillary tangles in the brain. The plaques are comprised of a β-amyloid peptide, which is produced when Amyloid Precursor Protein (APP) is cleaved by β-secretase (encoded by the *BACE* gene. Elevated oxidative metals can implement changes in learning and memory. We tested the role of protein expression in memory loss using the Aversive Phototaxis Suppression (APS) assay. Co-expressing the human APP and human BACE genes in the nervous system of *Drosophila* using the UAS ELAV/GAL4 system resulted in a ~40% decrease in pass rate, as compared to outcrossed controls. This decrease in short term memory demonstrates functional losses similar to those seen in AD patients. Altering copper levels in the AD model changed the severity of symptoms. This assay can be used effectively to test the role of other components implicated in AD pathogenesis, like tau and other oxidative metals. This could allow for further studies of mushroom bodies, functionally similar to hippocampi in humans and responsible for learning and memory, in the fly AD model. .

639B

The role of Synaptotagmin1 (SYT1) in a *Drosophila* model of MJD. Rachel Pearcy, John Warrick. University of Richmond, Richmond, VA.

Machado-Joseph's Disease (MJD) is a neurodegenerative, polyglutamine disease that currently has no effective treatment or therapy. The goal of this research is to determine how the disease pathology of MJD is affected by up regulating and down regulating the expression of synaptotagmin 1 (*syt1*) in a *Drosophila* model. In previous research, Syt1 levels were found to be decreased in mice and *Drosophila*that were expressing the mutant form of the protein associated with MJD (Ataxin-3) (Chou et al, 2008, Neurobiology of Disease). Because of this, it was hypothesized that increasing Syt1 levels might slow the process of neurodegeneration in diseased flies. Data collected from plastic sectioning experiments appear to support this idea since some of the degeneration in diseased flies was rescued by an upregulation of Syt1 in young flies. Current experiments are focused on trying to determine if the low levels of Syt1 caused by the disease affect neuronal transmission and if higher levels of Syt1 can remedy issues with neurotransmitter release caused

by the disease. If Syt1 were found to play an active role in the disease pathology, Syt1 and synaptic regulation would be a potential target for developing a therapy for this disease.

640C

Cisplatin Induces Behavioral and Mitochondrial Changes in the Absence of Apoptosis in *Drosophila* Larvae Motor Neurons. Jewel Podratz, Han Lee, Patrizia Knorr, Stephanie Koehler, Steven Forsythe, Kelsey Lambreckt, Kiley Schmidt, Gabrielle Steinhoft, Goshya Yudinsev, Amy Yang, Eugenia Trushina, Anthony Windebank. Department of Neurology, Mayo Clinic, Rochester, MN.

Cisplatin and oxaliplatin are highly effective in treating testicular and colon cancers, yet 20-30% of patients develop peripheral neuropathy. Cisplatin binds nuclear DNA and mitochondrial DNA (mtDNA) inducing DNA damage and apoptosis. DNA adducts inhibit mtDNA replication and transcription inducing mitochondrial degradation in dorsal root ganglion neurons, *in vitro*. *In vivo*, cisplatin induces neurotoxic climbing deficiencies in adult *Drosophila melanogaster* that is directly related to apoptosis in the fly brain neurons. Our studies were designed to look at the effects of cisplatin on mitochondrial dynamics in *Drosophila* larvae motor neurons. We used D42-MitoGFP flies, which express GFP (green fluorescent protein) in the mitochondria of motor neurons. Larvae were treated with 0, 5, 10, 25 and 50ug/ml cisplatin for 3 days and the larvae observed for survival and righting behavior. Larvae treated with 10ug/ml cisplatin had a survival rate of 82% with a 68% increase righting time and was the concentration used for our experiments. Motor neuron mitochondria were then examined for axonal trafficking, membrane potential and mitochondrial length in larvae treated with 0 and 10ug/ml cisplatin. Axonal trafficking showed a slight decrease in velocity of anterograde mitochondria and more frequent stopping with cisplatin treatment. However, there was no difference in mitochondrial length. Cisplatin decreased TMRM (tetramethylrhodamine methyl ester) uptake in GFP positive mitochondria by 27%. Immunostaining of motor neurons for active caspase 3 showed no apoptosis in 0 or 10ug/ml cisplain but when the concentrations was increased to 25ug/ml cisplatin, active caspase 3 was observed. These results show that 10ug/ml cisplatin induces righting deficiencies in *Drosophila* larvae, affects mitochondrial membrane potential and axonal trafficking in the absence of apoptosis. Together, changes in mitochondrial dynamics might be an early sign of cisplatin-induced peripheral neuropathy and mitochondria an important therapeutic target.

641A

Alternative Splicing of *Drosophila Nmnat* Acts As A Switch Between NAD Synthetic and Neuroprotective Functions. K. Ruan, C. Li, RG. Zhai. Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL.

Neurodegenerative diseases triggered by a variety of genetic, epigenetic, and environmental factors are increasingly prevalent worldwide. Maintaining neuronal homeostasis is a prerequisite for proper neurological activity. However, details of mechanisms of neuronal maintenance are largely unknown, and in particular it is unclear whether and how healthy neurons regulate their self-protective activities. Previous work in our laboratory and others has found housekeeping NAD synthetic enzyme NMNAT proteins to be among the most effective and versatile neuroprotective factors in *Drosophila* and mammals. Here we show that the *Drosophila Nmnat* gene is alternatively spliced into two mRNA variants, RA and RB, which leads to two sets of functionally distinct proteins: PA/PC from RA, with higher enzyme activity, and PB/PD from RB, with better neuroprotective capacity to protect against spinocerebellar ataxia 1 (SCA1)-induced neurodegeneration. Furthermore, under stress conditions, the RB variant is preferably spliced in neurons to produce the neuroprotective PB/PD protein variant. Our results indicate that alternative splicing acts as a switch in NMNAT protein expression between the NAD synthetic enzyme function and the neuroprotective factor function. Neurons use this switch mechanism to partition NMNAT into two distinct functions: NAD synthesis and neuroprotection. Neurons respond to stress by driving the switch to produce the neuroprotective variants, and therefore achieve self-protection. Our work reveals a key mechanism of how neurons achieve and regulate self-protection against adverse conditions.

642B

Role of Signaling Pathways in Aβ42 mediated neurodegeneration. A Sarkar[1], A Singh[1,2,3]. 1) Department of Biology, University of Dayton, Dayton, OH; 2) 2)Premedical Program, University of Dayton,300 College Park Drive, Dayton,OH; 3) 3)Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

Alzheimer's disease is an age related neurodegenerative disorder. Accumulation of the Aβ42 plaques is one of the vital reasons for AD mediated neurodegeneration. It has been suggested that Aβ42 plaques triggers oxidative stress due to impaired signaling, which result in neuronal cell death. However the exact mechanism causing cell death is still not well understood. We employ a *Drosophila* eye model of AD by misexpressing high levels of Aβ42 in the differentiating photoreceptors of the fly retina. Our aim is to discern the role of signaling pathways involved in neurodegeneration. In a forward genetic screen, we have identified *teashirt (tsh), crumbs* (*crb*) and other members of Wingless (Wg) signaling pathway as genetic modifiers of Aβ42 mediated neurodegeneration. It is known that *wg* is a negative regulator of differentiation in the eye. Our preliminary data suggests that by misexpression of Shaggy kinase (Sgg), a negative regulator of the Wg signaling pathway, suppress the neurodegeneration caused by Aβ42 misexpression. We will test the role of Wg signaling in Aβ42 plaques mediated neurodegeneration. Furthermore, we will analyze, if these modifiers act independent and/or parallel of each other or whether they have a linear relationship in triggering neurodegenerative response due to accumulation of Aβ42.

643C

The Effects of Copper on Larval Motility and Larval Learning and Memory in a Drosophila Model of Alzheimer's Disease. Courtney Schoff, Brie Paddock. Arcadia University, Biology Department, Glenside, PA, 19038.

One of the hallmark signs of Alzheimer's Disease is the presence of amyloid plaques within the brain and their accumulation is

associated with synaptic dysfunction. Amyloid beta is generated by the cleavage of Amyloid Precursor Protein (APP) by a series of proteins, include beta-secretase (BACE). Heavy metals have been implicated in the formation and accumulation of Amyloid beta, but the mechanism is unclear. Using third instar *Drosophila melanogaster* coexpressing human APP and human BACE in their nervous system and raised on food with varying levels of available copper. The distance crawled, number of body contractions and distance per contraction we measured. The appetitive associative olfactory learning was examined to determine neuronal dysfunction. The neuromuscular junctions in the larvae control the crawling behavior in Drosophila, which is thought to be compromised by high levels of heavy metals. The differences of this crawling behavior and learning and memory in the flies co-expressing hAPP and hBACE indicate deficiencies in synaptic function due to presence of copper. This could implicate that the hydroxyl radical formed from the reaction of heavy metals with hydrogen peroxide within the body as a factor that could cause Alzheimer's disease.

644A

Probing integrin signaling in neuronal maintenance in flies. Mumine Senturk[1], Shinya Yamamoto[2], Manish Jaiswal[3], Hugo Bellen[1,2,3,4]. 1) Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) HHMI; 4) Department of Neuroscience; Neurological Research Institute at Baylor College of Medicine, Houston, TX.

Integrins form strong adhesive junctions between tissue layers, a process that is required in the wing to keep the dorsal and ventral cells attached to each other. A loss of adhesion between these two epithelial layers causes wing blisters. Our lab generated a collection of essential *Drosophila* X chromosome mutants. To identify new players in the integrin signaling pathway, we screened these EMS induced mutations for wing blisters in mutant clones in the wing and thorax. We identified 30 new alleles of known integrin pathway genes and 27 additional mutants that exhibit similar wing blistering phenotypes. We mapped many of these mutants and one corresponds to *ubiquilin* (*ubqn*), the *Drosophila* homologue of *UBQLN*. Ubqn functions in the ubiquitin-proteasome system-mediated degradation by recruiting ubiquitinated proteins to the proteasome. Mutations in *UBQLN2* have been shown to cause dominant X-linked amyotropic lateral sclerosis (ALS) and mutations in *UBQLN1* have been linked to Alzheimer's disease (AD) by genetic linkage analysis and family-based association studies. We found that loss of ubqn in the fly eye causes a neurodegeneration, glial death, and an accumulation of small mitochondria upon aging. Moreover, young retina of the *ubqn* mutants display an ER expansion which is one of the distinguishing features of several mutants that cause ALS. Interestingly, several components of the integrin signaling pathway have recently been associated in GWAS for AD. The link between Ubqn, integrin signaling, and neuronal maintenance is intriguing and I am therefore exploring this link using *Drosophila* as a model system.

645B

The *atrogin-nutcracker* F-box protein family of genes in Drosophila models of degenerative disease. Brian Staveley, Eric Merzetti, Colleen Furlong, Poly Talukdar, Lindsay Dolomount, Maggie Hickey. Biology, Memorial Univ. of Newfoundland, St. John's, NL, Canada.

A large family of SCF ubiquitin ligases exists to add ubiquitin units as a method of post-translational modification of certain proteins. The protein substrates are specified through interaction with the F-box protein subunit. Here we chose to focus upon a small group of closely related F-box protein genes due to their association with human degenerative diseases. Defects in *Fbxo7* (or *PARK15*) has linked to neurodegeneration, specifically a rare early onset or juvenile form of Parkinson disease (parkinsonian-pyramidal disease) and *Fbxo32* (or *atrogin*) has been found to play a role in muscle degeneration in mammals by targeted degradation of muscle proteins, and through modified enhancement of *foxo* activity. Our goal is to model aspects of human degenerative disease through alteration of these genes in Drosophila. The Drosophila homologue of *atrogin* (*CG11658*), when overexpressed does not produce severe degenerative phenotypes although subtle defects in longevity, locomotor ability and eye development were observed. Overexpression of *atrogin* enhances *foxo* phenotypes, suggesting the conservation of the interaction between these genes. In Drosophila, loss of *Fbxo7* (or *nutcracker*) influences caspase activity to cause sterility (Bader *et al.,* 2010; 2011) and *Fbxo7* overexpression rescues *parkin* mutant degenerative phenotypes (Burchell *et al.,* 2013). In our studies, directed expression suppresses the *α-synuclein*-dependent (*SNCA*) phenotypes and RNA-inhibition of *Fbxo7* and its binding partner *PI31*, in the dopaminergic neurons reduces lifespan. The RNA-inhibition of the closely-related *Fbxo9*produces a slightly reduced lifespan. In summary, examination of these *Fbxo* genes will continue to provide insight into processes of degeneration. *Thanks to Dr. H. Steller for ntc & PI31 stocks. Funded by Memorial University TA's & Fellowships to EMM, CBF & PT and by NSERC Scholarships to CBF& MKH and an NSERC Discovery Grant to BES.*.

646C

A *Drosophila* Model of Radiation-Induced Neurological Damage. Lisa Sudmeier[1], Steven Howard[2], Barry Ganetzky[1]. 1) Dept. of Genetics, University of Wisconsin, Madison, WI; 2) Dept. of Human Oncology, University of Wisconsin SMPH, Madison, WI.

Children who undergo cranial radiation therapy (CRT) to treat central nervous system malignancies are at increased risk for neurocognitive deficits, impaired coordination and motor control, and seizure disorder. The severity of these side effects often worsens as age at treatment decreases. One strategy to reduce the negative sequelae of CRT is to identify therapeutic targets to enhance radioprotection in healthy tissue. To model radiation-induced damage during development, we irradiated *wild-type* third instar larvae, with a single dose (10, 20, 30, 40, or 50 Gy) of radiation from a Cesium-137 source and allowed them to develop to adulthood at 25°C. Developmental survival was calculated as the percentage of irradiated larvae at each dose that survived to adulthood. Motor function was examined in five-day-old adult flies by determining the percentage of flies that climb 5 cm or more in 10 seconds. Adult longevity was determined by assaying lifespan, and neuronal cell death was assessed by immunohistochemistry in adult brains. We also tested

developmental stage sensitivity to radiation by irradiating larvae at various ages. Irradiating late third instar larvae at a dose of 40 Gy or higher results in a precipitous reduction in the percentage of larvae that survive and develop into adults. A dose of 20 Gy or higher impairs motor activity of surviving adults. A dose-dependent decrease in adult longevity is paralleled by a dose-dependent increase in activated caspase in adult brains. Survival to adulthood and adult lifespan are more severely impaired with decreasing larval age at time of irradiation. We are currently screening the Drosophila Genome Reference Panel (DGRP) for quantitative trait locus (QTL) mapping of genetic variants that alter developmental survival and motor function following irradiation and have identified a number of candidate genes, which we are now testing for their ability to alter these radiation-sensitive phenotypes. This work demonstrates the usefulness of *Drosophila* to model the toxic effects of radiation during development with the potential to unravel underlying mechanisms and to discover novel therapeutic interventions.

647A

Investigating the cell type specific roles of Neuropathy Target Esterase using *Drosophila*. Elizabeth Sunderhaus, Sudeshna Dutta, Doris Kretzschmar. Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR.

Neuropathy Target Esterase (NTE) is a phospholipase that maintains lipid homeostasis and can regulate PKA activity. Mutations in NTE have been shown to cause a spectrum of disorders with symptoms ranging from hypogonadism to cerebellar atrophy. Loss of NTE's ortholog, Swiss Cheese (SWS), in *Drosophila melanogaster* has been shown to cause age-dependent neurodegeneration similar to the degeneration seen in the brain-specific knockouts of murine NTE when aged. To investigate how different mutations in NTE result in distinct phenotypes, we have created transgenic flies expressing wildtype and mutant human NTE (hNTE). Expressing wildtype NTE pan-neuronally in the genetic background of *sws*[1] showed that hNTE can suppress the neurodegeneration and rescue the decreased phototaxis performance and lifespan of the *sws*[1] mutant. However, SWS and NTE are also expressed in glia, and *sws*[1] has glial defects and cell death. Expressing hNTE in glia suppressed cell death, confirming that the construct can replace SWS, and we can test the functional ability of the mutant constructs in flies. For example, a hNTE construct without the phospholipase domain due to a frame shift mutation does not rescue degeneration when expressed in neurons, and we are currently testing its function in glia. Using a construct with a mutation in the active site of the phospholipase domain of SWS, we found that expression of this construct in glia or neurons cannot rescue degeneration, but it can rescue retinal degeneration when induced in the eye. In contrast, a construct with a mutation in the PKA interaction domain cannot rescue the neuronal degeneration, including photoreceptors, but can rescue glial death. This data shows that different cell types have different requirements for the two functions of SWS, suggesting that mutations in NTE specifically affect one of these functions, thereby causing specific phenotypes. We will therefore also test how the mutant hNTE constructs affect the phospholipase and PKA interaction domains' function. *Drosophila* can therefore provide a model to gain further insights into the functions of NTE and how disturbing the domains cause the spectrum of phenotypes seen in patients.

648B

Dysregulation of Dopamine Handling in Dopaminergic Neurons of *Drosophila* Parkinson's disease models. Antonio Tito[1,2,6], Shebna Cheema[3], Zhen Xu[2], Yanning Rui[2], Sonal Jaiswal[4], Zhihua Chen[2], Wen-Ting Li[5], Mian Jiang[3], Hugo Bellen[4,7], Sheng Zhang[1,2,6]. 1) Graduate Programs of Human Molecular Genetics & Neuroscience; 2) Institute of Molecular Medicine; 3) Department of Natural Sciences, UHD; 4) Program in Developmental Biology. BCM; 5) Department of Natural Sciences, Rice; 6) UT-GSBS. UT-Medical School. UTHEALTH; 7) Howard Hughes Medical Institute.

Parkinson's disease (PD) is characterized by the prominent loss of dopaminergic (DA) neurons in the Substantia Nigra, a region of the mesencephalon that plays an important role in motor control and goal-directed behaviors. Recent studies of *parkin* and *Pink1,* two genes responsible for autosomal-recessive juvenile form of PD (AR-JP), suggest a potentially important role of mitochondria dysfunction in PD pathogenesis. However, the molecular mechanisms underlying the relatively selective degeneration of DA neurons in PD are still unknown. Dopamine, the neurotransmitter synthesized by DA neurons, is highly unstable due to its unique chemical structure, prone to oxidation in a basic environment with concomitant production of toxic reactive oxidative species (ROS). Accordingly, dopamine itself has long been suspected to play a role in the etiology of PD. In DA neurons, dopamine is normally uptaken into the cell and sequestered into synaptic vesicles (SVs) by dopamine active transporter (DAT) and vesicular monoamine transporter (VMAT), raising a possibility that their dysregulation might contribute to the disease development. To test this hypothesis, we have generated MiMIC-based eGFP-tagging and Gal4-trap lines and carried out a detailed documentation of the *in vivo* expression and subcellular localization of *VMAT* protein. We have further characterized several UAS-VMAT transgenes and*vmat* mutant alleles for their gain- and loss-of-function phenotypes in whole animals and in DA neurons. Using these tools, we are currently determining the physiological effect of ex-vesicular dopamine on the survival of DA neurons in the brains of wildtype or *parkin* and *Pink1* mutant flies*,* and the resulting findings will be presented.

649C

Insights Into Ataxin-3's Neuroprotective Function. Wei-Ling Tsou, Sokol Todi. PHARMACOLOGY & NEUROLOGY, WAYNE STATE UNIVERSITY, DETROIT, MI.

Polyglutamine (polyQ) diseases are dominantly inherited neurodegenerative disorders caused by polyQ expansions in otherwise unrelated disease proteins. As there is currently no treatment for these devastating disorders, there is a pressing need to develop therapeutic strategies. Spinocerebellar Ataxia Type 3 (SCA3) is one of the nine known polyQ diseases. SCA3 occurs when there is an abnormal polyQ expansion in the deubiquitinating enzyme (DUB), ataxin-3. Wild type ataxin-3 has been shown by others and us to have a neuroprotective function in *Drosophila* models of polyQ degeneration. Ataxin-3's DUB activity and its own ubiquitination are required

for its neuroprotective effect. However, the molecular mechanism through which ataxin-3 performs its neuro-protective role is not clear. Here, we use *Drosophila* to examine the physiological importance of protein quality control processes in ataxin-3's protective role. We find that the previously reported cooperation of ataxin-3 with the E2 ubiquitin-conjugating enzyme Ubch5, Ube2W, and Ubc13, or with E3 ubiquitin-ligases CHIP, E4b/Ufd2, Parkin and HRD1 is not important for ataxin-3-dependent neuroprotection. Knockdown of autophagy genes does not affect ataxin-3's neuroprotection. However, the interaction of ataxin-3 with proteasome-associated factors is important for suppressing degeneration. According to other results, ataxin-3 is required both in the cytoplasm and the nucleus to suppress degeneration, and it performs this function without altering the overall protein levels of the disease protein. Biochemical studies indicate that ataxin-3 reduces degeneration in *Drosophila* by regulating toxic protein aggregation, rather than its turnover, probably by deubiquitinating aggregated species. Current experiments are further dissecting the pathways and components through which ataxin-3 reduces polyQ-dependent degeneration.

650A

Loss of *isocitrate dehydrogenase 3α* causes synaptic transmission defects in the fly visual system via reduced alpha-ketoglutarate levels. Berrak Ugur[1], Hector Sandoval[2], Nele Haelterman[1], Manish Jaiswal[2,4], Shinya Yamamoto[1,2], Hugo Bellen[1,2,3,4]. 1) Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics,Baylor College of Medicine, Houston, TX; 3) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 4) Howard Hughes Medical Institute.

To identify and characterize novel components in phototransduction and neurodegeneration, our lab has performed a large scale, clonal, forward genetic screen on the X chromosome of *D. melanogaster*for essential genes. The screen was based on the *ey*-FLP system to generate homozygous mutant eye clones in an otherwise heterozygous mutant background. The ability of homozygous mutant photoreceptors to respond to light was assessed in aging flies by recording ERGs (electroretinogram). From this screen, we isolated 4 different mutations in the fly homolog of *isocitrate dehydrogenase 3α*(*CG12233*). The protein that is encoded by *CG12233* contains a mitochondrial localization signal and belongs to the isocitrate and isopropylmalate dehydrogenase family. It functions in the Krebs cycle to catalyze the oxidative decarboxylation of isocitrate resulting in α-ketoglutarate (αKG) and CO_2. Homozygous mutant animals are viable till the third instar larval stage and they have reduced αKG levels. *idh3α* mutant photoreceptors show synaptic transmission defects, along with progressive, light-independent loss of ERG amplitudes, a readout for neurodegeneration. Interestingly, we found that feeding the*idh3α* eye specific mosaic flies with αKG rescues the synaptic transmission defect whereas it fails to rescue the progressive loss of ERG amplitude. We hypothesized that IDH3α is regulating synaptic transmission by controlling the neurotransmitter pool. We are currently analyzing the metabolome of *idh3α* mutant flies to underpin additional metabolites that may cause neurodegeneration upon loss of*idh3α*..

651B

Imaging of sub-synaptic LRRK translocation using a tagged genomic knock in, created by MiMIC replacement. Sven Vilain[1,2], Roeland Vanhauwaert[1,2], Sandra Fausia Soukup[1,2], Raquel da Cunha[1,2], Patrik Verstreken[1,2]. 1) VIB Center for the Biology of Disease, 3000 Leuven, Belgium; 2) KU Leuven, Department of Human Genetics and Leuven Research Institute for Neuroscience and Disease (LIND), 3000 Leuven, Belgium.

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. The most frequent cause of familial PD are mutations in the leucine-rich repeat kinase 2 (*LRRK2*), the orthologue of fly *dLRRK*. We have previously shown that Lrrk plays a role in synaptic vesicle cycling, if dLRRK plays a direct role in vesicle cycling it requires a presynaptic localization of the protein. In order to localize endogenous dLRRK, we created an HA-tagged *dLRRK* knock in allele by capitalizing on the availability of a MiMIC transposon carrying recombinogenic *attP* sites in the neighboring gene. We inserted a recombination cassette flanked by unique *I-SceI* and *I-CreI* restriction enzyme sites between the two MiMIC *attP*-sites and used I-SceI and I-CreI mediated double strand breaks to remove unwanted flanking transposon sequences, while leaving a triple *HA* tag sequence before the *dLRRK* stop codon. $dLRRK^{HA}$ is fully functional and reveals synaptic localization of the tagged dLRRK-HA protein. We are now using this tool to assess the synaptic distribution of the protein and we are assessing localization of the protein in response to low and high frequency stimulation paradigms. We hope these studies will help to reveal the normal function of dLRRK at synapses in healthy but also diseased synaptic terminals. .

652C

Modeling TRPV4-mediated Hereditary Axonal Neuropathies in *Drosophila*. Brian M Woolums, Morgan Yang, Catherine Mamah, Jeremy Sullivan, Amanda Le, Charlotte Sumner, Thomas Lloyd. Johns Hopkins School of Medicine, Baltimore, MD.

Three distinct hereditary axonal neuropathies are caused by dominant missense mutations in the gene encoding TRPV4, a Ca^{2+}-permeable cation channel of the TRP superfamily of ion channels. In patients with TRPV4 mutations, the clinical phenotype is notable for distal denervation, suggesting that mutant TRPV4 protein causes motor axonal degeneration. Here, we apply a combination of *in vitro* and *in vivo* approaches to examine the pathogenic mechanisms underlying TRPV4-mediated neuropathies. Functional studies demonstrate that expression of mutant TRPV4 in cultured cells causes increased channel activity, calcium influx, and cellular toxicity suggesting a gain-of-channel function. To investigate mechanisms of mutant TRPV4-mediated neurodegeneration *in vivo*, we have generated transgenic*Drosophila* expressing the neuropathy-causing mutant $TRPV4^{R269C}$. Pan-neuronal expression of $TRPV4^{R269C}$ results in mutation-specific phenotypes, including a failure of adult wing expansion, a phenotype also observed following selective expression of $TRPV4^{R269C}$ in CCAP neurons (N_{CCAP}), and can be rescued by genetic inactivation of the TRPV4 channel pore, demonstrating the dependency of the phenotype on channel activity. Expression of $TRPV4^{R269C}$, but not $TRPV4^{WT}$, in N_{CCAP} causes adult-onset axonal

swellings and accumulations of dense core vesicles. Furthermore, expressing $TRPV4^{R269C}$, but not $TRPV4^{WT}$, in larval dendritic arborization (da) neurons results in axonal swellings and aberrant synapses, and these phenotypes are most pronounced in neurons projecting to posterior segments. Both adult and larval phenotypes suggest $TRPV4^{R269C}$ may impair axonal transport. We have conducted an RNAi-based screen for genetic modifiers of $TRPV4^{R269C}$ neurotoxicity and have identified calcium-calmodulin dependent protein kinase II (CaMKII), a known regulator of the cytoskeleton, as a potent suppressor of $TRPV4^{R269C}$ mediated neurotoxicity. We are currently characterizing CaMKII and other modifiers from our screen in hopes of gaining insight into the mechanisms of TRPV4-mediated neurotoxicity. .

653A

α-Synuclein interacts with *VPS35* in an improved *Drosophila* model of Parkinson's Disease. Hui Ye[1,2], Amit Chouhan[1,2], Puja Yogi[1,2], Elizabeth Kowalis[1,2], Shreyasi Chatterjee[3], George Jackson[3], Joshua Shulman[1,2]. 1) Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 3) University of Texas Medical Branch, Galveston, TX.

Parkinson's Disease (PD) is neuropathologically defined by cytoplasmic inclusions of aggregated α-synuclein protein (αSyn), in association with dopaminergic neuronal loss within the midbrain substantia nigra. Previously, αSyn transgenic flies were shown to recapitulate neurodegeneration, but the overall levels of toxicity were modest. In order to refine the *Drosophila* PD model for genetic modifier screening, we engineered a codon-optimized, human αSyn transgene and targeted mature photoreceptors using the *Rh1-GAL4* driver. We achieved at least 5-fold increased expression of αSyn compared to previously available lines, and observed a correspondingly enhanced toxicity profile with exquisite age- and dose-dependence. Our model demonstrates early synaptic pathology, based on histologic degeneration in the lamina and electroretinogram (ERG) transient potential changes consistent with defective neurotransmission. With aging, progressive destruction of lamina and retinal tissue is seen, culminating in cell loss and reduced phototransduction by ERG. Importantly, retinal degeneration can be monitored using the pseudopupil preparation in intact, unfixed heads, facilitating genetic interaction screening. In a survey of selected Mendelian PD genes, we found that knockdown of *VPS35* using RNAi or available mutant alleles potently enhanced αSyn neurotoxicity *in vivo*. Similar interactions were also observed with *VPS26* mutants, suggesting a broader role for the retromer in synucleinopathy. We are currently examining the functional impact of the PD-associated $VPS35^{D620N}$ mutation, including activity in our improved αSyn transgenic model. We are also expanding the scope of our interaction screening to other PD susceptibility genes. In sum, our results link the *VPS35* gene with αSyn-mediated mechanisms and establish an improved *Drosophila* PD model as a powerful tool for future investigations. .

654B

Loss of *Nardilysin*, a mitochondrial peptidase, causes a slow progressive neurodegeneration and affects the Krebs cycle by modulating oxoglutarate dehydrogenase activity. W.H. Yoon[1,5], H. Sandoval[1], M. Jaiswal[1,5], S. Jaiswal[1], S. Yamamoto[1,2,6], N. Putluri[4], V. Putluri[4], A. Sreekumar[4], T. Donti[1], B. Graham[1], H.J. Bellen[1,2,3,4,5,6]. 1) Molecular and Human Genetics; 2) Program in Developmental Biology; 3) Department of Neuroscience; 4) Department of Molecular and Cellular Biology, Baylor College of Medicine; 5) Howard Hughes Medical Institute; 6) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

We isolated mutations in *Drosophila Nardilysin* (*dNrd1*), a novel mitochondrial peptidase, that cause a progressive loss of neuronal function. NRD1 was previously shown to localize to the cytoplasm, nucleus, and plasmamembrane. We found that fly and vertebrate NRD1 contain mitochondrial targeting sequences that target both to mitochondria. Both mitochondrial localization and peptidase activity of dNRD1 are required for its *in vivo* function. Interestingly, *dNrd1* mutants do not display a decrease in membrane potential or ATP level. To elucidate the role of NRD1 in mitochondria, we performed IP/MS using a V5-tagged dNRD1. dNRD1 preferentially interacts with enzymes of the Krebs cycle. Co-IP confirmed that NRD1 interacts with oxoglutarate dehydrogenase (OGDH), a key enzyme in the Krebs cycle. To test whether loss of *dNrd1* affects the Krebs cycle metabolism, we perform metabolomics of metabolites in the Krebs cycle in *dNrd1* mutants. Loss of *dNrd1* exhibits an accumulation of alpha-ketoglutarate (a-KG). These results suggest that OGDH requires dNRD1 for its function since OGDH use a-KG as a substrate and a decrease in OGDH activity can lead to an accumulation of a-KG. We find that loss of *dNrd1* causes a dramatic decrease in the level and activity of OGDH. To test whether OGDH is a major target of dNRD1 in neural protection, we took advantage of the MiMIC/DegradFP system. This permits conditional knock-down of OGDH protein in photoreceptor neurons. Knock-down of OGDH phenocopy neuronal dysfunction observed in *dNrd1* mutants. Hence, dNRD1 is a novel mitochondrial protein that is required for neuronal protection by conferring stability to a critical enzyme in the Krebs cycle. Since loss of *Nrd1* in mice has been shown to cause a peripheral neuropathy and aberrant fat metabolism, we are currently studying patients with point mutations in *Nrd1*. .

655C

Expansion of polyglutamine repeats in huntingtin perturbs the motility of Rab4 and Rab11 containing vesicles in Drosophila larval axons. Katherine Zimmerman, Joseph White, Dr. Shermali Gunawardena. University at Buffalo, Buffalo, NY.

Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of polygluatmine repeats in the huntingtin (HTT) gene that results in the death of stiatal neurons. Patients with polyQ lengths >35 are at risk of getting the disease. Previous work in HD models showed that reducing HTT levels caused axonal transport defects. While the cargo that HTT transports is unknown, recent work has shown that reduction of HTT levels in *Drosophila* dramatically perturbs the movement of both YFP-Rab4 and Rab11-GFP *in vivo* suggesting that HTT is required for the normal transport of those Rab proteins. Using *in vivo* analysis, we characterized how expansion of polyQ repeats in HTT affected the motility of these Rab proteins. We found that while HTT 72Q and HTT 138Q both

caused large axonal accumulations (blocks), HTT 15Q showed robust motility. Further, while both Rab4 and Rab11 motility was perturbed by expansion of polyQ repeats, HTT co-localized with Rab4 and Rab11 axonal blocks. Together our data suggests that expansion of polyQ repeats in the context of HTT found in HD disrupts the normal movement of Rab4 and Rab11. Rab4 or Rab11 may form a complex with HTT during proper transport.

656A

Compensatory signaling mechanisms related to Parkinson's disease in a fly model of dopamine loss. Karol Cichewicz[1], Emma Garren[1], Martin Darvas[2], Jay Hirsh[1]. 1) Biology Department, University of Virginia, Charlottesville, VA; 2) Pathology, University of Washington, Seattle, WA.

Parkinson's disease (PD) is a neurodegenerative disorder primarily affecting motor functions due to death of dopaminergic (DA) neurons. First symptoms of the disease do not arise until 70-80% of DA neurons die, suggesting that poorly described compensatory mechanisms play a role in delaying symptoms. We developed a genetically modified strain of *Drosophila* lacking DA in the central nervous system (CNS) that developed a behavioral suppression of the locomotor activity loss during repeated backcrossing and selection. Although the initial outcrossed DA-deficient line shows severely reduced locomotor activity, by selecting individuals with extreme phenotypes we were able to establish two stable sublines, one with low activity and another one with surprisingly normal locomotor activity, now called 'DA Bypass', both without DA in the CNS. We propose that the surprising rescue of locomotor activity in this brain DA-deficient line may be related to compensatory mechanisms in presymptomatic PD. We are investigating this behavioral phenomenon using brain transcriptome and quantitative trait locus (QTL) techniques. To determine SNP's responsible for the DA Bypass phenotype, we will utilize bulk segregant analysis, an efficient whole genome sequencing-based technique used to identify QTLs. We are conducting an RNA-SEQ analysis comparing transcriptomes expressed in our DA deficient strains and wild type controls. Using a similar mouse DA-deficiency model (TH:DAT KO) (Henschen et al. 2013), we are conducting an RNA-SEQ to compare mouse pre- and postsynaptic DA brain regions. By comparing both datasets we hope to identify signaling mechanisms activated in the absence of DA that can compensate for the loss of this neurotransmitter.

657B

Functional Studies of Spermine Synthase and Establishing a *Drosophila* Model for Snyder-Robinson Syndrome. C. Li, C. Bello, R. Zhai. Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL.

Snyder-Robinson Syndrome (SRS) is a condition characterized by intellectual disability, seizures, bone abnormalities, and movement disorders. SRS was reported to have an X-linked recessive inherited pattern and the causative gene was mapped to the spermine synthase gene (SMS). Spermine synthase is a highly conserved enzyme and is involved in polyamine synthesis. Polyamines, including putrescine, spermidine, and spermine are small, positively charged molecules that are essential for numerous cellular processes such as DNA synthesis, transcriptional regulation, and modulation of ion channel activities. However, the functions of SMS and polyamine in the nervous system are unclear and there is no treatment for SRS. Our results show that *Drosophila* SMS is expressed in the central nervous system and loss of SMS disrupts polyamine homeostasis in the *Drosophila* brain. We found SMS mutant flies have severe climbing performance defect and reduced life span. Specifically, we found that loss of SMS function causes an age-dependent synaptic degeneration with abnormal ultrastructure in SMS mutant synapses, suggesting a specific role of SMS and polyamines in maintaining synaptic integrity. Our work will not only benefit the understanding and potential therapeutic design for SRS, but also reveal the essential function of polyamines in nervous system in general.

658C

***KCNT1* in Epilepsy with ID and Psychiatric Features: Modeling Human Disease in *Drosophila*. Chiao Xin Lim**[1,2], Sarah E. Heron[1,2], Michael G. Ricos[1,2], Leanne M. Dibbens[1,2]. 1) School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, SA, Australia; 2) Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, Australia.

Epilepsy has a global prevalence of 2%. Approximately 60% of all epilepsy cases are known to be genetic in origin. Mutations in a number of different ion channel genes are associated with seizure phenotypes. Mutations in the sodium-activated potassium channel subunit gene, *KCNT1*, cause two different seizure disorders. We have reported *KCNT1* mutations in a severe form of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), which includes intellectual disability (ID) and psychiatric features. In ADNFLE patients experience violent motor seizures during sleep. Mutations in KCNT1 have also been found in cases of very severe early onset epilepsy associated with cognitive impairment (epileptic encephalopathy) known as malignant migrating focal seizures of infancy (MMFSI). To study the mechanisms underlying the seizure phenotypes caused by *KCNT1* mutations, we used antisense RNA to knockdown *KCNT1* expression in *Drosophila*. Our results showed that reduced expression of*KCNT1* in *Drosophila* neurons using an *Elav-GAL4-UAS-Dicer2* driver and UAS-RNAi led to a seizure phenotype in flies when tested using the bang-sensitivity behavioural assay. In addition, we are generating transgenic flies which will carry a number of the recurrent point mutations we have found in epilepsy patients. These include mutations that cause ADNFLE alone (Arg928Cys), mutations that are found exclusively in patients with MMFSI (Gly288Ser), and mutations that are associated with both ADNFLE and MMFSI (Arg398Gln). These transgenic flies will allow us to perform ectopic expression of the mutated KCNT1 protein using the GAL4/UAS system, and provide us with insights into whether a loss of activity, increase in activity or altered activity of the KCNT1 channel protein is contributing to the different types of epilepsy. This system will serve as a model for testing novel therapeutic interventions. .

659A

Adenosine suppresses seizure-like activity (SLA) and shortens recovery time in the Bang-sensitive (BS) paralytic mutants following mechanical shock. Chris Radlicz, Daniel Kuebler. Department of Biology, Franciscan University of Steubenville, Steubenville, OH.

The *Drosophila* Bang-sensitive (BS) paralytic mutants exhibit seizure-like activity (SLA) following a variety of insults and have proven to be a good model system for investigating both the etiology of seizure disorders as well as identifying potential anti-convulsant therapies. For example, recent studies have demonstrated that metabolic and dietary alterations can suppress SLA in the BS mutants. Given that extracellular adenosine is a key neuromodulator that couples metabolic stress with a reduction in neural excitability, we investigated the ability of adenosine to suppress SLA in the BS mutants. The BS mutants *easily-shocked (eas)* and *technical knockout (tko)* were fed 1 gram of standard media supplemented with 200 mg of adenosine for three days. In both cases, the adenosine fed flies exhibited reduced SLA following a mechanical shock. The distance the adenosine fed *tko* flies moved during the bout of SLA and the total time they spent exhibiting SLA were reduced by over 50%. A similar but smaller reduction was seen in adenosine fed *eas* flies. In addition, the adenosine fed *tko* flies recovered more than 25% faster from mechanical shock than control tko flies; 77±5.2 sec vs. 109±8.1 sec. Taken together, these data indicate that adenosine, a compound known to suppress seizures in mammalian seizure models, appears to have a similar anti-convulsant effect in *Drosophila*. .

660B

A Drosophila model of human DYT dystonia. Noriko Wakabayashi-Ito[1], Rami Ajjuri[2], Benjamin Henderson[2], Olugbenga Doherty[2], Xandra Breakefield[1], Janis O'Donnell[2], Naoto Ito[1]. 1) Neurology, Massachusetts General Hosp, Boston, MA; 2) Biological Science, University of Alabama, Tuscaloosa, AL.

Dystonia represents the third most common movement disorder in humans with over 20 genetic loci identified. *TOR1A (DYT1)*, the gene responsible for the most common primary hereditary dystonia, encodes torsinA, an AAA ATPase family protein. Most cases of DYT1 dystonia are caused by a 3 bp (GAG) deletion that results in the loss of a glutamic acid residue (ΔE302/303) in the carboxyl terminal region of torsinA. This torsinAΔE mutant protein has been speculated to act in a dominant-negative manner to decrease activity of wild type torsinA. *Drosophila melanogaster* has a single torsin-related gene, *dtorsin*. Null mutants of *dtorsin* exhibited locomotion defects in third instar larvae. Levels of dopamine and GTP cyclohydrolase (GTPCH) proteins, which is encoded by *Punch*, were severely reduced in *dtorsin*-null brains. Further, the locomotion defect was rescued by the expression of human torsinA or feeding with dopamine. Here, we demonstrate that human torsinAΔE dominantly inhibited locomotion in larvae and adults when expressed in neurons using a pan-neuronal promoter Elav. Dopamine and tetrahydrobiopterin (BH_4) levels were significantly reduced in larval brains and the expression level of GTPCH protein was severely impaired in adult and larval brains. When human torsinA and torsinAΔE were co-expressed in neurons in *dtorsin*-null larvae and adults, the locomotion rates and the expression levels of GTPCH protein were severely reduced. These results support the hypothesis that torsinAΔE inhibits wild type torsinA activity. Similarly, neuronal expression of a *Drosophila* DtorsinΔE equivalent mutation dominantly inhibited larval locomotion and GTPCH protein expression. These results indicate that both torsinAΔE and DtorsinΔE act in a dominant-negative manner. We also demonstrate that Dtorsin regulates GTPCH expression at the post-transcriptional level. This *Drosophila* model of DYT1 dystonia provides an important tool for studying the differences in the molecular function between the wild type and the mutant torsin proteins.

661C

Dg-Dys-Syn1 signaling via microRNAs acts in a feedback loop to control the precision of Dystroglycan expression that is required for proper brain formation. Mariya M. Kucherenko, Andriy S. Yatsenko, April K. Marrone, Halyna R. Shcherbata. MPRG Gene expression and signaling, Max Planck Institute for biophysical chemistry, Goettingen, Lower Saxony, Germany.

Dystrophin-glycoprotein complex (DGC) is often associated with fatal inherited neuromuscular disorders. Major DGC components are evolutionary conserved from flies to humans, making *Drosophila* a good model for studying the DGC function and regulation. Using *Drosophila* muscular dystrophy (MD) model, we found that stresses accelerate the onset of MD in DGC mutants and can induce similar symptoms in *wt* animals. miRNAs are good candidates to act as stress response factors as they allow for a quick cellular response. We analyzed miRNA profiles in dystrophic and *wt* animals under normal and stress conditions. We grouped identified miRNAs into those linked to dystrophy and/or stress. Stress-related miRNAs are of particular importance as they reveal more general role of DGC in cellular homeostasis regulation compromised by stress. It has been shown in vertebrates that a pathway involving DGC and Syn-NOS signaling regulates histone modifications that modulate transcription of multiple genes, including miRNAs. We showed that DGC-Syn-NOS signaling also exists in flies. Particularly, during brain development, it regulates expression of the *miR-310s* complex, which in turn can target Dystroglycan (Dg), the main DGC component. This allowed us to propose a novel perceptive-executive model for protein level adjustment: it is perceptive, because it senses high levels of a critical protein, and it is executive, because it results in transcriptional activation of miRNAs that target the protein to restore its normal levels. This regulation is important for stabilization of Dg levels, since aberrant Dg amounts or *miR-310s* and NOS signaling deficiencies result in cobblestone brain, resembling human lissencephaly type II phenotype. As a consequence of *miR-310s* regulation, only a portion of Dg mRNAs with an extended *3'-UTR* can be targeted, preventing Dg levels from being reduced below a certain threshold, which also is damaging for brain formation. Thus, the variability of *3'-UTR* sequences has great significance in regulation of gene expression, where miRNAs act as managers of this extra layer of precision.

662A

Fluorescent Imaging of the Drosophila Melanogaster Model of Human Nephrolithiasis. Sohrab Naushad Ali, Dajung Kim, Thomas Tailly, Hassan Razvi, Hon Leong. Division of Urology, University of Western Ontario, London, Ontario, Canada.

PURPOSE: Drosophila Melanogaster (DM), a powerful translational model for many human diseases has recently emerged as a viable model of human nephrolithiasis. We have developed a novel bone and hydroxyapatite- specific fluorescent probe, Alendronate-FITC along with its negative control, Notdronate-FITC, for imaging calcium containing stones *in vitro* and *in vivo*. We investigated the utility of these probes in studying calcium oxalate stone formation in DM malpighian tubules (MT). METHODS: Wild type DM were reared in a climate controlled room, temperature 22-25° C, humidity 40% and a 12 hour light/dark cycle. They were divided into groups and fed either a standard corn meal based diet or lithogenic diets containing 0.5% (w/v) Ethylene glycol or 0.5% (w/v) Sodium Oxalate. At 3 weeks DM were sampled from each group, the MT's dissected and incubated with Alendronate-FITC, Notdronate-FITC and PBS as a control. The MT's were then imaged using a confocal microscope. RESULTS: DM fed the sodium oxalate diet showed more consistent stone formation compared to DM fed the ethylene glycol diet. DM fed the standard diet and stained with Alendronate-FITC showed scattered fluorescence in the anterior MT, most likely due to staining of its natural calcium concretions. DM fed either of the lithogenic diets showed concentrated fluorescence throughout the MT, indicating the presence of calcium oxalate stones when compared to *in vitro* stained hydroxyapatite nanoparticles stained with Alendronate-FITC. DM fed lithogenic diets and stained with Notdronate-FITC showed weak fluorescence with no signal in the lumen of MT's. CONCLUSIONS: Calcium oxalate stones can successfully be imaged using bisphosphonate based fluorescent probes. The superiority of these probes over conventional imaging techniques lies in the fact that they can be used for non-invasive *in vivo* imaging of stone formation. DM diets containing Alendronate-FITC may be used for imaging and quantifying the effects of different therapies on stone formation.

663B

Role of sodium-phosphate co-transporter *MFS13* in *Drosophila* phosphate homeostasis. Clemens Bergwitz[1,2], Jonathan Cohen[2], Mark Wee[2], Dana Drost[1]. 1) Medicine/Section Endocrinology, Yale School of Medicine, New Haven, CT; 2) Endocrine Unit, Massachusetts General Hospital, Boston, MA.

We recently described a member of the major facilitator superfamily (MFS) of transporters in *Drosophila*, which is expressed predominantly in the Malpighian tubules and gut (PLoS One 7: e31730, 2012). This transporter, *FBgn0010497/MFS13*, is related to yeast *Pho84* and human *SLC17A1-9*, an anion transporter subfamily with members known to mediate phosphate transport. When expressed in*Xenopus* oocytes *MFS13* mediates uptake of [^{33}P]-orthophosphate in a sodium-dependent fashion. RNAi-mediated knockdown in fly hemocyte S2R+ cells, which endogenously express *MFS13*, inhibits phosphate-induced phosphorylation of rolled/ERK/MAPK. We observed that fly blood phosphate and calcium-phosphate deposits in the distal tips of the Malpighian tubules increase over life time in flies on high phosphate diet. Supplementation with 1% sevelamer to prevent absorption of phosphate from the diet reduces both blood phosphate and tubule deposits. To begin to evaluate the role of *MFS13* in fly phosphate homeostasis, we used transgenic RNAi lines to reduce expression of this transporter universally, or selectively in gut or in the Malpighian tubules. We also generated *MFS13* hypomorphic flies using p-element rescue. Loss of *MFS13* expression does not affect larval development. However, adult flies display increased hemolymph phosphate levels when *MFS13* is ablated universally, but not, when ablated selectively in Malpighian tubules or in gut. qRT-PCR suggests that transporter mRNA is induced by dietary phosphate in the gut. These findings suggest that *MFS13* negatively regulates hemolymph phosphate and calcium-phosphate deposit formation in Malpighian tubules, which is currently subject of further investigation in the lab.

664C

Role of cellular phosphate uptake for *Drosophila* life span. Clemens Bergwitz[1,2], Jonathan Cohen[2], Mark Wee[2], Dana Drost[1]. 1) Section Endocrinology/Dept. Medicine, Yale School of Medicine, New Haven, CT; 2) Endocrine Unit, Massachusetts General Hospital, Boston, MA.

Phosphate is required for important cellular processes and hypophosphatemia causes rickets and osteomalacia in humans. Hyperphosphatemia, on the other hand, leads to reduced lifespan due to tissue calcifications and metabolic changes by mechanism(s), which are to date poorly understood. We used *Drosophila melanogaster* as a model organism and showed that excess dietary phosphate raises fly blood phosphate, causes tissue mineralization and reduces adult fly life span. A similar lifespan reduction is seen when excretion of phosphate is genetically reduced. Conversely, addition of sevelamer to reduce absorption of phosphate from the diet or phosphonoformic acid (PFA) to block cellular uptake of phosphate extends adult fly life span (PLoS ONE 8(3): e56753, 2013).
To better understand, whether the cellular response to phosphate is required for life span reduction in hyperphosphatemic flies, we used transgenic RNAi lines and the gene-switch system to reduce expression of the phosphate transporters *FBgn0260795/dPit* and *FBgn0010497/MFS13* in all fly tissues when treated with mifepristone. This raises fly blood phosphate and results in tissue mineralization, however, longevity of these flies was unaffected or improved, when compared to flies cultured in the absence of mifepristone on a high phosphate medium or when compared to wild type flies cultured on control medium. Our data suggest that phosphate must bind the transporters and/or enter cells to reduce life span. The lab is currently exploring the role of the MAPK pathway, which is activated by phosphate in cells of many multicellular organisms and known to reduce lifespan in yeast and in mice.

665A

Drosophila Models of Genetic Renal Diseases. Margaret Nettleton, Fujian Zhang, **Zhe Han**. Center for Cancer and Immunology Research, Children's National Medical Center, Washington, DC.

We have discovered that the Drosophila heart also functions as a kidney. The cardiac nephrocyte combines the signature features of human glomerular podocytes and renal proximal tubules for filtration and protein reabsorption. From a large-scale genetic screen for genes required for nephrocyte functions, we have identified over a hundred renal genes, including fly homologs of about 20 most known renal disease genes. When compared to the exome-sequencing data from renal disease patients, our data helped to reveal three novel renal disease genes (ADCK4, ARHGDIA and KANK2) and the underlying molecular mechanisms caused by these mutations. We also developed Drosophila models carrying the exact genetic mutations identified from renal disease patients and tested candidate drugs that could rescue the renal defects in these disease models. Our studies demonstrate that Drosophila can be used as an ideal model system to study renal diseases and to perform drug discovery.

666B

A fly model to identify new treatments for hyperuricemia, uric acid stones and gout. S. Lang[1], H. Lu[1], T. Chi[2], A. Kahn[1], D. Killilea[3], M. Stoller[2], P. Kapahi[1]. 1) Kapahi Laboratory, Buck Institute, Novato, CA; 2) Department of Urology, University of California San Francisco (UCSF), San Francisco, California; 3) Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute, Oakland, California.

In the course of human evolution a multistep mutational event disrupted the uricase gene, whose gene product is the major uric acid metabolizing enzyme. With this deficiency, uric acid became the end product of the human purine degradation pathway and systemic uric acid concentrations rose to levels close to and sometimes in excess of the water solubility of uric acid (≥ 6mg/dl). Although the benefit of this adaption to higher uric acid levels is still controversial (e.g. increasing blood pressure to allow human upright walk), elevated uric acid levels often have clear disease manifestations related to pathological biomineralization including gouty arthritis and uric acid kidney stones. The RNAi mediated inactivation of uricase gene expression in *Drosophila melanogaster* phenocopies the hallmarks seen in many hyperuricemic patients. Besides elevated levels of uric acid and the formation of uric acid enriched stones/crystals in the excretory system (Malpighian tubule and hindgut) the disease phenotype is - as predicted - further augmented by feeding diets rich in purine. Moreover, the validity of the model was demonstrated by reducing the disease phenotype with an FDA approved inhibitor of uric acid formation, allopurinol. We are currently utilizing this novel fly model for a drug and genetic screen to identify a) compounds and b) novel molecular players as alternative and potentially more effective treatment strategies for gout and uric acid kidney stones. As proof of principle, we identified drugs (e.g. methotrexate) and target genes (e.g. PRPP synthetase) which act upstream of uric acid formation to prevent its synthesis and thereby mitigate the disease phenotype of our hyperuricemic fly model.

667C

Toxicological and development effects of polymer-coated iron oxide nanoparticles in Drosophila. Evan A. Chavers[1], Hunter B. Dean[1,2], Ben W. Henderson[1], Rami R. Ajjuri[1], Yuping Bao[2], Janis M. O'Donnell[1]. 1) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Department of Chemical Engineering, University of Alabama, Tuscaloosa, AL.

Polymer-coated, metal oxide nanoparticles have become increasingly relevant to biomedical research. As the clinical uses for nanoparticles expand, so, too, does the need for a comprehensive understanding of their toxicology. Because of the *in vitro* toxicity of comparable cobalt, nickel, and copper oxides, much of the focus has shifted to iron oxide nanoparticles (IONPs). However, much of the nanoparticles' toxicity can be attributed to the choice of polymer coating. Past research has largely been conducted in rodent models or *in vitro* using mammalian cell lines. There remains a need for a practical, whole-organism model to study nanoparticle toxicology *in vivo* and assess the immune activity and reproductive effects. Using *Drosophila melanogaster,* this study focuses on evaluating the toxic effects of polyacrylic acid (PAA)-coated and polyethylenimine (PEI)-coated IONPs. Second instar larva were fed PAA-coated or PEI-coated IONPs for 24 hours and assayed for larval development, pupation, eclosion, fertility, and immune response. Interestingly, we found that exposure to low concentrations (0.5-10µg/mL) of PAA-coated IONPs resulted in greater larval mortality than exposure to higher concentrations. Additionally, low-dose exposure caused reproductive defects in adults leading to a reduction in fertility but not embryo viability. When investigating reproductive tissue morphology, we found abnormalities in the ovarian cells of adults that had been dosed with PAA-coated IONPs as larvae. Additionally, we have identified a dose-dependent threshold of IONP exposure required to activate the immune response, which may explain the recovery of survivorship at higher-dose treatments. In larvae treated with the same concentrations of PEI-coated IONPs, no effects were found on larval survivorship, pupation, or eclosion. However, dose-dependent effects were observed in fertility of both sexes. Altogether, our work is consistent with previous studies showing differential effects between polymer coatings. We also noted a concentration threshold of immune system activation under polymer-coated IONP exposure.

668A

Analysis of the mechanism of action of the anti-migration/anti-metastatic compound Dihydromotuporamine C, using a leg imaginal disc eversion assay. Corey Seavey[1], Minpei Wang[1], Aaron Muth[2], Otto Phanstiel IV[3], Laurence von Kalm[1]. 1) Department of Biology, University of Central Florida, Orlando, FL; 2) Department of Chemistry, University of Central Florida, Orlando, FL; 3) Department of Medical Education, College of Medicine, University of Central Florida, Orlando FL.

Cancer chemotherapeutics with good anti-migration/anti-metastatic activities are significantly underrepresented in the arsenal of anti-cancer agents. One compound with excellent anti-migration properties in mammalian cell culture is dihydromotuporamine C (Motu 3,3)

that was isolated from a sea sponge near Motupore Island, New Guinea. Motu 3,3 has been reported to activate RhoA and to influence sphingolipid metabolism in mammalian cells. Unfortunately, the mechanism of Motu 3,3 toxicity in cell culture is not currently understood. Using a leg imaginal disc eversion assay we have shown that Motu 3,3 acts through Rho1 in Drosophila cells in a pathway leading to activation of the myosin non-muscle heavy chain (Zipper). In an attempt to determine the mechanism of toxicity and potentially separate toxicity and anti-migration activities we have tested derivatives of Motu 3,3 for their ability to activate Rho1 and inhibit leg eversion in vitro. These data are compared directly to assays with Motu 3,3 derivatives in mammalian cell culture. .

669B

A *Drosophila* model to address genetic mechanisms preventing age-related muscle fiber loss. Lizzet Castillo, Richard Cripps, Anton Bryantsev. Department of Biology, University of New Mexico, Albuquerque, NM.

The persistent decline in overall counts of individual fibers is a persistent feature found in aging human muscles, which is thought to contribute to the pathogenesis of aging-related muscle wasting (sarcopenia). Identification of the wide range of genetic factors influencing muscle resistance against aging-related fiber loss constitutes an important task in the development of therapeutic approaches to combat sarcopenia. The jump muscle of the fruit fly *Drosophila melanogaster* faithfully reproduces the morphologic and functional features characteristic of aging human muscles. Specifically, aging*Drosophila* jump muscles lose individual fibers and demonstrate progressive impairment in the ability to generate power for jumping. We have developed a model to study aging-related fiber loss in flies. This model can be effective in genetic screening to identify critical factors affecting muscle susceptibility to fiber loss.

670C

Representing Drosophila Models of Human Disease in FlyBase. P. McQuilton[1], S. Tweedie[1], G. Millburn[1], A. Schroder[2], J. Goodman[3], H. Attrill[1], S. Marygold[1], L. Ponting[1], R. Stefancsik[1], N. Brown[1], FlyBase Consortium. 1) FlyBase-Cambridge, Department of Genetics, University of Cambridge, Cambridge, CB2 3EH. United Kingdom; 2) FlyBase-Harvard, Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138. USA; 3) FlyBase-Indiana, Department of Biology, Indiana University, 1001 East 3rd Street, Bloomington, Indiana 47405-7005. USA.

Drosophila is used extensively to model a wide variety of human diseases, particularly neurological diseases, and the number of papers describing these models is increasing year-on-year. Since the beginning of 2014, **FlyBase** has been curating Drosophila models of human disease with the aim of making these data accessible to the whole range of researchers, from Drosophila specialists to clinicians. Disease model annotations are made by assigning terms from the Disease Ontology (disease-ontology.org) to alleles shown to recapitulate an aspect of a human disease. These alleles may be mutations or transgenic constructs of Drosophila genes, or human genes expressed in Drosophila. In addition, alleles/transgenes that exacerbate or ameliorate the disease model are also recorded. As of September 2014 (FB2014_05) FlyBase includes nearly 2,500 disease annotations, from around 500 references. These are composed of 126 different diseases (approximately two-thirds of which are neurological) and are attached to over 1,500 alleles from more than 750 genes. Here, we will explain how to search FlyBase for these disease annotations and describe how they are displayed in the new 'Human Disease Model Data' section of the relevant Gene and Allele Reports in FlyBase.

671A

Investigating the mechanism of basement membrane repair and its implications in wound healing. William Ramos-Lewis[1], Erica Shannon[1], Andrea Page-McCaw[1,2]. 1) Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Cancer Biology, Vanderbilt University, Nashville, TN.

We are utilizing a Drosophila 3rd instar epidermal wounding model to investigate the mechanism of basement membrane repair, and its influence on re-epithelialization. The basement membrane is a specialized sheet of extracellular matrix that lies on the basal surface of epithelial cells, providing structural support and signaling regulation to the surrounding tissue. The protein composition of basement membrane varies between tissues, but the major components found in all basement membrane include laminin, collagen IV, nidogen, and the heparan sulfate proteoglycan perlecan. Although studies of basement membrane date back to the early 1900s, many question surrounding its repair and role in wound healing remain unanswered. Drosophila offers a plethora of genetic tools to interrogate these fundamental processes. Specifically, we are using this toolkit to determine if the re-incorporation of specific basement membrane proteins regulates re-epithelialization during the wound healing process and, if so, to elucidate the mechanisms. Furthermore, to develop this model as a means to interrogate the mechanism of basement membrane repair, we are currently mapping the sources of each epidermal basement membrane component during repair. Preliminary data suggests that collagen IV in the epidermal basement membrane originates from the fat body, which secretes soluble collagen IV into the hemolymph, during both normal growth and repair. We speculate that basement membrane repair is important for normal wound closure. To test this hypothesis, we are using tissue specific drivers to knock down basement membrane components via RNAi, prior to wounding, and evaluating the response of epidermal cells at the level of cell migration, polarization, and signaling. .

672B

The role of *Drosophila clueless* in regulating mitochondrial morphology. Zong-Heng Wang[1], Erika Geisbrecht[1,2]. 1) School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO; 2) Department of Biochemistry and Biophysics, Kansas State University, Manhattan, KS.

Mitochondria are dynamic organelles regulated by opposing fusion and fission processes, governed by Marf/Mitofusin and Drp1, respectively. *clueless* (*clu*) is well known for its role in the prevention of mitochondrial clustering. However, it is unclear how Clu controls

mitochondrial distribution and if it affects mitochondrial fusion/fission. In this study, we utilized the *Drosophila* larval and adult musculature as models to examine mitochondrial dynamics. In wild-type (WT) msucles, mitochondria are elongated and tubular. However, they are swollen in *clu* mutant or *clu* RNAi muscles and appear fragmented upon Clu overexpression (OE). Knockdown of *marf* by RNAi results in round, smaller mitochondria, while the mitochondria in *drp1* RNAi muscles are fused into longer, tubular structures. Strikingly, Clu OE in*marf* RNAi exhibits more mitochondrial fission, but Clu OE in *drp1* RNAi muscles restores the mitochondrial morphology close to WT. It is well-known that Pink1 and Parkin facilitate the degradation of Mitofusin on damaged mitochondria to promote mitophagy. Here, we found that mitochondrial clustering is enhanced in *clu, parkin* double mutants. Both Pink1 and Parkin OE can restore mitochondrial swelling in *clu* RNAi. However, Clu OE only partly rescues mitochondrial shape in *pink1* mutants, but not in *parkin* mutants, suggesting *clu* is parallel to *parkin*. To corroborate this, TUNEL labeling shows that *clu* mutant muscles do not undergo cell death as in *parkin* mutant muscles. Further, our submitted paper showed that Clu interacts both genetically and physically with *Drosophila* dGrasp65 to mediate export of αPS2 but not βPS integrin from perinuclear ER in larval muscles by suppressing ER stress. Both ER stress and abnormal accumulation of αPS2 in *clu* RNAi can be mitigated by feeding larva with chemical chaperones. However, the drug feeding failed to rescue the mitochondrial swelling in *clu* RNAi. Taken together, Clu acts upstream or in parallel to the Pink1-Parkin pathway and interacts with fusion and fission genes to regulate mitochondrial morphology, independent of its role in integrin secretion.

673C

***In silico* analysis of recombination rate variation across the *Drosophila melanogaster* genome based on multiple DNA motifs. Andrew Adrian**, Josep Comeron. Biology, University of Iowa, Iowa City, IA.

In all eukaryotic species examined, meiotic recombination and crossovers (CO) in particular occur non-randomly along chromosomes though the molecular causes for this distribution remain largely unexplained. Studies in mice and humans have identified a single motif (a 13-bp motif that is the binding site of the protein PRDM9) that is highly enriched in CO hotspots, but even in this case the presence of the PRDM9 motif is a very poor predictor of CO localization along chromosomes. In Drosophila, where CO distribution also varies extensively along chromosomes, no PRDM9 homolog exists. In fact, many motifs have been identified to be enriched in regions containing recombination events. Here, we present a genome-wide study of these recombination-enriched motifs in *D. melanogaster* and investigate whether variation motif presence can be used to infer variation in recombination rates across the genome. Using the Random Forest (RF) learning algorithm for modeling, we have identified a subset of motifs that have a significant effect on recombination localization when in concert, showing that these motifs are indeed not only enriched around a subset of recombination events but have also significant predictive power to describe recombination variation at a genome-wide scale. Our results do not confirm that in Drosophila multiple motifs can contribute to the initiation or resolution of recombination events but rather that primary–sequence data add a non-negligible layer of genomic context determining CO localization.

674A

The effect of spatially varying selection on transposable element insertions in Drosophila. Jeffrey Adrion[1], David Begun[2], Matthew Hahn[1]. 1) Department of Biology, Indiana University, IN; 2) Department of Evolution and Ecology, UC Davis, CA.

Natural populations often experience environmental and spatial variation in the strength and targets of natural selection over their ranges. This spatially varying selection can drive adaptation to the environment and consequently shape patterns of polymorphism across the genome. Drosophila provides an excellent opportunity to study the effects of spatially varying selection in natural populations, as both *D. melanogaster* and *D. simulans* have recently (within the last 500 years) been introduced into North America, having since colonized the bulk of both continents. Previous studies have identified candidate single nucleotide polymorphisms (SNPs) that are potential targets of spatially varying selection, and have described broad patterns of SNP variation along clines in both North America and Australia. Here, we investigated how spatially varying selection impacts another important source of genomic variation, transposable elements (TEs). TE insertion dynamics shape genome evolution and, in multiple instances, have been shown to be the causative mechanism underlying adaptation to the environment. We detect novel TE insertions in whole-genome pooled-population sequence data sampled from eight populations of *D. melanogaster* and nine populations of *D. simulans* spanning a latitudinal cline on the east coast of North America. We analyze TE insertion dynamics and characterize variation in the distribution and frequency of individual TEs and TE families, as well as interactions between TEs and other features of the genome, such as piRNA clusters. Our results shed light on how TE insertions shape genome evolution in Drosophila and identify insertions that may underlie environmental adaptation.

675B

Annotation of *Drosophila biarmipes* contigs from euchromatic chromosome 3 as a control for the heterochromatic dot chromosome. Rachel Boody, Alexander Kneubehl, Lauren Guerriero, Jamie Sanford. Biological & Allied Health Sciences, Ohio Northern University, Ada, OH.

Chromosome 4 in *Drosophila,* commonly referred to as the dot chromosome, is distinct from other autosomes in that it is highly heterochromatic. Remarkably, despite its heterochromatic composition, the dot chromosome has a similar proportion of transcribed genes as compared euchromatic chromosomal regions. The Genomics Education Partnership (GEP) of Washington University, St. Louis, seeks to understand the evolutionary mechanisms behind this uncommon gene expression. Using a primarily undergraduate work force, the GEP employs comparative genomics to analyze homologies between various newly sequenced *Drosophila* species. Comparative analyses of dot chromosome gene structure and control euchromatic chromosome 3 will help to elucidate the

evolutionary mechanisms that may lead to transcription of genes in the heterochromatic dot chromosome. The present study focuses on the annotation of contigs 13 and 52 from the third chromosome of *D. biarmipes*. As these contigs are on a euchromatic chromosome, they serve as a control for the heterochromatic dot chromosome. Data from annotation of contig 13 from chromosome 3 of *D. biarmipes* revealed the presence of several gene homologs: CG7369, l(3)04053, CG11241, CG32454, CG11367, CG14450. Annotation of contig 52 revealed Syn1, Rbp8, and 10 putative single exon gene homologs. Results were obtained using a series of bioinformatics approaches, including NCBI Blast, Flybase Blast, and the UCSC Genome Browser. Gene models were developed based on collected data, and consist of intron and exon coordinates for each isoform of each gene homolog. Future work will expand upon these gene models through determination of transcription start sites and motif hunting to identify DNA regulatory sequences that may contribute to gene expression in the dense heterochromatic landscape of the *Drosophila* dot chromosome. .

676C

Genome evolution in three species of cactophilic Drosophila. Fernando Peñaloza[1], **Javier Carpinteyro Ponce**[2], Nestor Nazario Yepiz[2], Mariana Ramirez Loustalot-Laclette[2], Luciano Matzkin[3], Cei Abreu Goodger[2], Alejandro Sanchez Flores[1], Therese Markow[1,4]. 1) Unidad Universitaria de Apoyo Bioinformatico, Instituto de Biotecnologia, UNAM, Cuernavaca Morelos, Mexico; 2) LANGEBIO, CINVESTAV Irapuato, Irapuato, Guanajuato, Mexico; 3) Department of Biological Sciences, The University of Alabama Huntsville, Huntsville, AL; 4) Division of Biological Sciences, UCSD, La Jolla, CA.

Three species of cactophilic flies form the mojavensis cluster: which includes the species *Drosophila mojavensis*, *D. arizonae* and *D. navojoa*. *Drosophila navojoa,* an opuntia breeder, is basal to the other two species, which have switched to columnar cactus hosts in their respective ranges. Fixed inversions in chromosome 1, 2 and 3 characterize the species. Despite of their close evolutionary relationships and their sympatric distribution for some populations, hybrids have not been found in the wild. The three species provide an excellent model to study speciation and adaptation events. Although the ecology and physiology of these species have been studied for several years, it is still poorly understood what is occuring at genomic level. Here we present the genomes for *D. arizonae* and *D. navojoa*and an updated genome annotation for *D. mojavensis*. We sequenced and assembled the genomes for *D. arizonae* and *D. navojoa* using both Illumina HiSeq 100bp paired end and mate pair libraries We used the genome of *D. mojavensis* as a reference to orient contigs and assign them to Muller elements. We generated transcriptome data for the three species using Illumina HiSeq 100bp paired end libraries to perform gene model predictions and annotations for the three genomes. According to our results the 146 Mb genome of *D. arizonae* and 115 Mb genome of *D. navojoa* encondes 12100 genes and 10000 genes respectively. We performed protein sequence comparison and clustering to define shared and unique gene families between the three species and estimated the dN/dS ratios over the three genomes. Finally we identified the transposable elements distributed over the three genomes.

677A

Population genomic analysis of the infectious and integrated *Wolbachia pipientis* genomes in *Drosophila ananassae*. Jae Young Choi, Jaclyn Bubnell, Charles Aquadro. Cornell University, Ithaca, NY.

Coevolution between *Drosophila* and its maternally inherited bacterial endosymbiont *Wolbachia pipientis* has many intriguing aspects. For example, *Drosophila ananassae* hosts two forms of *W. pipientis* genomes where one originates from the infectious bacterial genome, and the other is found integrated into the host nuclear genome. Here, we have characterized the genomics of the infectious and integrated genomes of *W. pipientis* in *D. ananassae* (wAna), by whole genome sequencing of several strains of *D. ananassae* from around the world that have either the infectious or the integrated forms of the wAna genomes. Results indicate evolutionarily stable maternal transmission for the infectious wAna genome since the species' initial infection that we have estimated to have occurred 210,000 generations ago. In contrast, the integrated wAna genome shows pseudogene-like characteristics with an accumulation of many nucleotide substitutions and structural variants that are predicted to have deleterious effects if present in an actively transcribed and/or infectious bacterial genome. Computational analysis of the host-genomic locations of the integrated wAna genome predict a major insertion site in the hosts' 2ndchromosome, which was commonly found in all *D. ananassae* strains with the integrated genome and consistent with the initial discovery of a *W. pipientis* insertion in *D. ananassae*. Additional integrations (potentially of only subsets of the wAna genome) were also discovered in other autosomal and X chromosomal locations, and these integrations are polymorphic among the *D. ananassae* strains that harbor the integrated genome. Surprisingly, our analysis of the copy number and segregating variants of the integrated wAna genome indicates the presence of at least two complete wAna genomes integrated into the host genome in all strains of sampled *D. ananassae* that contain an integration. The integrated genomes' polymorphic presence in diverse geographical populations of *D. ananassae* raises two intriguing questions: 1) what drove the wide geographical dispersal of the initial integration, and 2) why does this double *W. pipientis* integration persist given that it causes a >1% increase in the total genome size of *D. ananassae*?

678B

Evolutionary Analysis of the *Drosophila* Sex Peptide Network. Meaghan McGeary, **Geoffrey Findlay**. Department of Biology, College of the Holy Cross, Worcester, MA.

The sex peptide (SP) network in *Drosophila* consists of at least 13 reproductive proteins from males and females that interact to control female behavior and physiology after mating. Function of this network is necessary for reproductive success. We are investigating the mechanisms of molecular evolution of each protein in the SP network. We determined the full-length coding DNA sequences for each SP network protein in the original 12 *Drosophila* species sequenced, and in 10 newly sequenced species. We then used PAML to identify sites and lineages that have evolved under positive selection, revealing that at least five of the network proteins

have evolved adaptively. Three-dimensional structural modeling identified specific regions of these proteins under selection. We have also observed that several of these genes are lineage restricted, implying piece-wise evolution of this network. These studies demonstrate pervasive adaptive evolution in a pathway implicated in male-female sexual conflict and make specific predictions about the functional and physical interactions between the members of this protein network. .

679C

Evolutionary and functional characterization of saturn, a newly evolved testis-expressed protein required for fertility in *Drosophila melanogaster*. Anna Gubala[1], Tery Vinh[1], Mariana Wolfner[2], Geoffrey Findlay[1,2]. 1) Department of Biology, College of the Holy Cross, Worcester, MA; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

De novo genes are genes of recent origin that are not explicitly related to ancestral coding sequences. Recent results in *Drosophila* show that the proteins these genes encode are often required for male reproductive function. We have identified a gene called *saturn* that encodes a small, testis-expressed protein with no detectable sequence homology to other proteins. Comparative genomics identified orthologs of *saturn* only in the melanogaster group of *Drosophila*. Molecular evolutionary analyses show that the saturn protein has evolved rapidly: several sites have evolved under positive selection, and we detected substantial variation in protein length in the highly disordered C-terminal region. Using several independent RNAi lines, we knocked down *saturn* in the germline of *D. melanogaster* males and observed a 70-90 percent reduction in male fertility. To investigate the fertility defect, we used knockdown males with GFP-labeled sperm. We observed that these males produce sperm that are motile and can be successfully transferred to the female fly, but that cannot become stored in the sperm storage organs of the female. These results highlight the dynamic turnover of proteins involved in reproduction and are consistent with previous reports that essential male reproductive proteins frequently arise from non-coding DNA sequence.

680A

***P-element* invasion and the evolution of host repression.** Yichen Zheng, Ricardo Azevedo, **Erin Kelleher**. Biology and Biochemistry, University of Houston, Houston, TX.

Transposable elements (TEs) are genomic parasites that pose a significant threat to their host. Transposition is a mutagenic process that can abrogate gene function, and ectopic recombination between homologous TE insertions introduces deleterious rearrangements and genome instability. Despite these costs of TE infection, existing theory suggests that host repression will rarely evolve in sexually reproducing organisms, because recombination and independent assortment rapidly separate repressors from the benefit of a reduced mutational load. In direct contradiction to this prediction, small RNA-mediated silencing pathways recently have been demonstrated to restrict TE propagation in eukaryotic genomes, revealing that host-repression evolves frequently, and through a conserved genetic mechanism. The evolution of host repression must therefore be reexamined in light of this new mechanism. We used the historical invasion of the *D. melanogaster* genome by the *P-element*, an autonomous DNA transposon, to explore the evolution of host repression. The *P-element* invaded the *D. melanogaster* genome in ~1950, and has subsequently driven the evolution of host repression in many natural populations. Recent studies suggest that *P-element* repression is predominantly enforced by the piRNA pathway, a germline-specific small RNA mediated silencing pathway. We exploited the extensive literature on the *Drosophila* piRNA pathway and *P-element* transposition to develop a realistic model of genome invasion and host genome response. We observed that for intermediate and high transposition rates, host repression evolves adaptively through both piRNA-dependent and -independent mechanisms. We furthermore observed that the selective advantage of piRNA-mediated repressors is amplified when repression reduces ectopic recombination between *P-element* insertions, as has been suggested to occur through piRNA-induced heterochromatin formation. Our findings suggest that adaptive evolution of host repression may be more common than previously appreciated, and provide a framework for examining the evolution of *P-element* repression in natural populations.

681B

The *Responder* satellite as a model for satellite DNA evolution in *Drosophila*. Daniel E Khost, Amanda M. Larracuente. University of Rochester, Rochester, NY.

With rapid, low cost sequencing becoming widely available, we have learned that the average eukaryotic genome is mostly comprised of non-coding, non-regulatory sequence. One class of this non-coding sequence is satellite DNA, which consists of simple or complex motifs repeated in long tandem arrays, and is usually found near the centromeres of chromosomes. There has been much debate over whether such sequences represent functional adaptations, or are simply the result of selfish processes that provide no benefit to their host genomes. Due to technical constraints, it has been extremely difficult to directly study these regions, as most current sequencing and assembly methods are inadequate for highly-repetitive, low complexity regions. However, recent advances in sequencing technology have enabled us to meaningfully explore the origin and possible functions of these previously inaccessible areas of the genome. We use the Responder (Rsp) satellite in Drosophila species as a model to examine fundamental properties of satellite DNA structure, activity, and evolution. Using high-coverage PacBio sequencing, we provide a detailed structure of a Rsp locus in the D. melanogaster 2R heterochromatin at a direct sequence level, and examine the mutational and recombination processes that shape its organization and evolution. From the map we have created for the locus, we use small RNA datasets to examine the transcriptional activity of Rsp. We find that it is differentially regulated between tissues and developmental timepoints, and we attempt to determine a mechanism for the production of Rsp small RNA.

682C

The role of *piRNA*-mediated epigenetic silencing in the population dynamics of transposable elements in *Drosophila melanogaster*. Grace Yuh Chwen Lee. Ecology and Evolution, University of Chicago, Chicago, IL.

The *piwi*-interacting RNAs (*piRNAs*) are small RNAs that target selfish transposable elements (TEs) in Drosophila genomes. Until now, *piRNAs'* role in TE population dynamics has only been discussed regarding their suppression of TE transposition, which alone is ineffective in preventing the constant increase in TE copy number. On the other hand, euchromatic TEs can be epigenetically silenced via*piRNA*-dependent heterochromatin formation and, similar to the widely known "Position-effect variegation", heterochromatin of TEs can "spread" into nearby genes. We hypothesized that the spread of*piRNA*-mediated heterochromatin of TEs to adjacent genes has deleterious functional consequences and leads to selection against individual TEs. Unlike other previously studied deleterious effects of TEs, which are due to TEs' physical disruptions of DNA, our proposed functional impact of TEs is mediated through their epigenetic influences. We found that the repressive chromatin mark, H3K9me, is elevated in sequences adjacent to euchromatic TEs. Furthermore, the heterochromatic status of genes depends not only on the number of and distance to adjacent TEs, but also on the probability that their nearest TEs are targeted by *piRNAs*. These variations in chromatin status likely have functional consequences, causing genes near TEs to have lower expression. Importantly, we found stronger selection against TEs that lead to higher H3K9me enrichment of adjacent genes, demonstrating the pervasive evolutionary consequences of TE-induced epigenetic silencing. Because *piRNAs* are generated and amplified through a "ping-pong cycle", the *piRNA*-dependent epigenetic impact of TEs likely depends quadratically on TE copy number. This can result in synergistic epistasis of TEs' deleterious effects, which has been theoretically shown to be a key requirement for the stable containment of TE copy number. Supporting this hypothesis, our forward simulations found that, without other mechanisms removing TEs, selection against *piRNA*-dependent epigenetic impact of TEs can lead to stably contained TE copy number. We are preforming additional simulations to understand the critical role of *piRNA*-mediated epigenetic silencing in the evolutionary dynamics of TEs.

683A

Diversity of short sequences among genomes of inversion strains in *Drosophila pseudoobscura*. Megan Lee[1], Sandra Duggan[2], Dianhui Zhu[2,3], Stephen Richards[2], Stephen Schaeffer[1]. 1) The Pennsylvania State University, University Park, PA; 2) Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, Houston TX 77030; 3) Chevron, 1500 Louisiana St, Houston, Texas, 77002.

The third chromosome of *Drosophila pseudoobscura* is polymorphic for over 30 paracentric inversions in western North American populations. The right arm of the X chromosome has two different gene arrangements in populations, while the other chromosome arms are monomorphic for gene arrangements in populations. Why some chromosomes are variable in gene arrangements and others are not is not well understood. Here, we analyze short repeat sequences of complete genomes that differ in third chromosomal inversion strains of *D. pseudoobscura* to determine if there are arrangement specific sequences. We used a computational approach to estimate the frequencies of all dimer to 10-mer sequences in each genome. The frequencies of dimer to 10-mer sequencess were estimated. The frequencies were ranked from largest to smallest in each strain and rank order correlations of motifs between pairs of different chromosomal arrangements of *D. pseudoobscura* were estimated In general, the frequencies of dimers to 10-mers were highly correlated among inversion strains with the exception of comparisons with the Pikes Peak inversion strain, which showed some motifs that differed dramatically in frequency to the compared arrangement. This alignment free approach to the study of sequence divergence suggests that the Pikes Peak strain has accumulated arrangement-specific short sequence motifs.

684B

Recurrent gene duplication of a rapidly evolving suppressor of transposable elements. Mia Levine[1], Emily Hsieh[1], Helen Vander Wende[1], Harmit Malik[1,2]. 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute.

Genetic conflict between a host genome and its transposable elements can promote evolutionary innovation. Host genes that suppress selfish activity in the germline frequently harbor a signature of adaptive protein evolution. The female germline-restricted *HP1D/rhino* locus is a classic example. Previous work demonstrated that *HP1D/rhino* accumulates amino acid-changing codons extremely rapidly. Here we report that HP1D/rhino also recurrently duplicates across the Drosophila melanogaster and montium subgroups. Using a combination of phylogenomic and synteny analysis, we discovered that at least eight structurally diverse, *HP1D/rhino*-derived daughter genes and "granddaughter" genes emerged during this 15 million year history. We address the functional significance of this prolific gene duplication and retention using P-element excision to generate a hypomorphic mutation in one daughter gene, *oxpecker*. We demonstrate that like its parent, *HP1D/rhino,* the 15 million-year old *oxpecker* supports female fertility in *D. melanogaster*. Using RNA-seq on wildtype and mutant ovaries, we discovered that loss of *oxpecker* expression in the female germline results in de-repression of several transposable element classes. We propose that *oxpecker* supports silencing of transposable element families incompletely suppressed by *HP1D/rhino*. These data implicate gene family expansion as an important evolutionary mechanism of genome defense diversification.

685C

***D. pseudoobscura* Inversion Breakpoint Sequence Evolution. Haley Randolph**[1*], Megan Lee[1], Geovanny Montoya[1], Atousa Jahanshahi[1], Sandra Duggan[2], Dianhui Zhu[2,3], Stephen Richards[2], Stephen Schaeffer[1]. 1) The Pennsylvania State University, University Park, PA 16801; 2) Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, Houston TX 77030; 3) Chevron, 1500 Louisiana St, Houston, Texas, 77002.

The chromosomal arrangements located on the third chromosome of *D. pseudoobscura* provide an exceptional system in which to study chromosomal arrangement evolution and the mechanisms that generate chromosomal inversions. All of the arrangements exist because of a series of overlapping paracentric inversions located in the noncoding regions of the third chromosome. As a continuation of previous research, the sequences of two pairs of inversion breakpoints, Santa Cruz to Chiricahua and Santa Cruz to Treeline, were assembled and analyzed. Previously, the Hypothetical to Standard, Standard to Arrowhead, and Standard to Pikes Peak arrangements were determined and analyzed and demonstrated that the inversion breakpoints occurred in noncoding sequences. Three kilobase large-insert paired-end libraries were utilized to map the breakpoints; genes that flanked the breakpoints were utilized as the beginning of the sequence assemblies. Once the breakpoint sequences were constructed, the ancestral and derived arrangements were used to determine the location of the chromosomal breaks. In each of the arrangements, areas of heavily-repeated DNA sequences are found, further suggesting that repeats play a role in the mechanism of chromosomal inversion creation. Similar repeats are found in the arrangements previously studied.

686A

Effect of sex biased expression or regulation of transposable elements on the *Drosophila melanogaster* genome. Christopher Savell, Brian Counterman. Biological Sciences, Mississippi State University, Starkville, MS.

Transposable elements have been shown to contribute to the regulation of gene expression through RNA interference and have recently been implemented in sex determination in *Bombyx*. To determine if differential expression of TEs in male and female germ lines may drive patterns of genomic divergence we examined the distribution of TEs across the X and autosomes in *Drosophila melanogaster*. First we determined the ratio of X to autosome copies for 78 TE families. Comparisons of the TE distributions and synonymous substitutions rates (ds) for over 2000 genes between *D. melanogaster* and *D. yakuba*, revealed similar mean values for dS and TE X/A ratios (~1.10). This suggests there is no male-bias on mutation rates and TE insertions in the *Drosophila* genome. In addition, we compared the X/A ratio of TE families known to be differentially expressed in ovaries and testes, to the of X/A ratio distribution for all other TE families, however no clear trend or significant differences were found. Unexpectedly, there were several TE families with X/A ratios at or above two, however there is no evidence that these TE families show differential expression in male and female germ line or that they are associated with small RNAs that are known to be differentially expressed in ovaries and testes of *Drosophila*. Currently we are examining the recombination rates of TEs with high X/A rations to determine if differences in recombination rate may be responsible for the excessive number of TE copies on the X chromosome in these TE families.

687B

Evolutionary aspects of gene expression during *Drosophila melanogaster* spermatogenesis. Júlia Raíces, **Maria Vibranovski**. Genetics and Evolutionary Biology, University of Sao Paulo, Sao Paulo, SP, Brazil.

New genes can arise by several different mechanisms [1]. Although some of those new genes are functional, most of them become pseudogenes. However, when they turn to be functional, new genes can quickly convert to essential genes or bear an important function at different phases of the development [2]. In this way, new genes expressed during spermatogenesis – the system of male gamete development - are probably related to fertility, mobility, form and function of the sperm cells. And, therefore, must be more expressed during the late phases of the gamete development, bearing in mind that expression relates to functionality. Hence, this project aims to test the hypothesis that there is a relation between a gene's age and its expression during spermatogenesis. According to this hypothesis, it's expected that new genes are more frequently expressed during post-meiosis, the latest phase of germline development. To answer those questions, bioinformatics and computational biology tools were used and proper statistical methods were applied to correlate already available data of gene age [3] and gene expression during spermatogenesis phases in Drosophila [4]. Our results in *Drosophila* have shown that the proportion of new genes expressed in late expermatogenesis (meiosis and post-meiosis) is significantly higher then in the beginning of the processes (mitosis). Also, we found that the expression level of new genes is higher than the expression of old genes during meiosis and post-meiosis, and the opposite pattern occurs during mitosis. These results implicates that new genes have an important role during the late spermatogenesis, which could be related to sperm fertility and speciation process. [1] Long *et al.* (2003). *Nature reviews* 4: 865-87 [2] Zhang *et al.* (2011). *PLoS Biol* 9(10): e1001179. [3] Zhang *et al.* (2010). *Genome Res.* 20: 1526– 1533. [4] Vibranovski *et al.* (2009). *PLoS Genetics,* 5:e1000731.

688C

Genetic architecture of parallel evolution of dark pigmentation in high-altitude populations of *Drosophila melanogaster*. Héloïse Bastide, Amir Yassin, John Pool. Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI.

In *D. melanogaster* the genes underlying well known phenotypic mutants found in the laboratory were thoroughly characterized over the years and yet, the connection between naturally segregating polymorphisms, phenotypic variation and the causative ecological factors is still lacking. We quantified abdominal pigmentation for 30 populations, mostly from sub-Saharan Africa. Ultra-violet intensity was the environmental variable most predictive of dark pigmentation in Africa, with highly melanic phenotypes observed in high-altitude populations of Ethiopia. However, the intensity of pigmentation also differs between African populations found on different mountains. A general consensus is that African populations of *D. melanogaster* show a higher magnitude of among-population genetic variation in nature, and thus constitute an ideal area for the study of parallel evolution of dark pigmentation in high-altitude populations. In order to unravel the genetic variation responsible for such a phenomenon, we crossed several parental lines collected from various dark and light African populations and established independent artificial populations in the laboratory. After several generations allowing a large number of recombination events, we mapped the variation underlying different pigmentation phenotypes

by combining bulk segregant analysis, pool-sequencing of the most extreme dark and light individuals, and population genomic data. We found a diversity of pigmentation traits varying among crosses, and uncovered distinct pigmentation genes strongly associated with the phenotypic differences between dark and light populations. Our results thus demonstrate the diversity of genetic mechanisms underlying convergent adaptations and the different ecological forces driving their evolution.

689A

Genetic Variation in Local Populations of *Drosophila suzukii*. Matthew DiMeglio[1], Philip Freda[1,2], **John Braverman**[1]. 1) Saint Joseph's University, Philadelphia, PA; 2) Kansas State University, Manhattan, KS.

Drosophila suzukii (Matsumura)**,** an invasive pest species known as spotted wing Drosophila, successfully expanded its range throughout the continental United States in only a matter of years, not unlike the geographical history of its close relatives, *D. melanogaster* and *D. simulans*. This parallel presents an opportunity to document the pattern of migration and test for insipient population structuring. This study, then, targeted the very recent colonization of the middle-to-north Atlantic, focusing on the greater Philadelphia area. *D. suzukki* were sampled from four geographic locations in the Northeastern United States. The population of *D. suzukii* in Lower Merion, PA, USA, was sampled over three years to test for changes over time. A 709-bp portion of *cytochrome oxidase subunit I* (*COI*) was amplified and sequenced from a total of 60 individuals. Nucleotide diversity was low in all populations sampled, however, haplotype diversity was high. F_{ST} values were low between geographic populations, consistent with a young population. Haplotype diversity and nucleotide diversity increased from 2011 to 2012 in Pennsylvania then fell slightly in 2013. To increase the sensitivity of the methods, 12 short tandem repeat (microsatellite) loci were identified using bioinformatics techniques. Primers for these loci are included in the results as a technical resource to others. Surveys revealed substantial polymorphism (n=10 male flies collected in 2014). These findings are consistent with very recent establishment of *D. suzukii* in the sampled populations.

690B

Hybridization and the spread of *Wolbachia* through space and time in the *yakuba* complex of the *Drosophila melanogaster* subgroup. Brandon S. Cooper, Paul Ginsberg, Chenling Antelope, Michael Turelli. Evolution & Ecology, University of California, Davis, Davis, CA.

Three species—*Drosophila yakuba*, *D. santomea*, and *D. teissieri*—comprise the *yakuba* complex within the *D. melanogaster* subgroup. While it was thought that only crosses between *D. yakuba*and its sister species *D. santomea* could produce fertile female hybrids, recent work has shown that all possible crosses between these species—including those with *D. teissieri*—produce fertile females and sterile males. This matches observed patterns of genomic variation where all three species share identical mitochondrial genomes despite nuclear differentiation. All three species have been reported to be infected with the same *Wolbachia* variant, supporting the hypothesis that regular introgression followed by backcrossing has led to shared cytoplasms among these three species; but the population biology of*Wolbachia* within this clade has not been thoroughly examined. By evaluating large population samples collected over the last ten years in continental Africa, and the islands of Bioko and São Tomé, we show that all species share the same *Wolbachia* variant and that infection frequencies vary through time and space. Infection frequencies seem to be increasing through time in all species, and the increase is statistically significant in *D. yakuba*. While *Wolbachia* infections in these species have previously been reported to not induce cytoplasmic incompatibility (CI), we have found segregating variation for CI in*D. teissieri*, and are evaluating whether *Wolbachia* increase fecundity and other components of fitness. We are also evaluating whether interactions with nuclear loci or with the abiotic environment can explain changes in infection frequencies, and variation in reproductive manipulations induced by the same *Wolbachia* variant. These data indicate that we are far from understanding the factors that maintain and change *Wolbachia* infection frequencies through time and space.

691C

Recombination rate variation among *Drosophila melanogaster* populations based on population genomic data. J. Cruz Corchado[1], J. Comeron[1,2]. 1) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, IA.

Recombination is a crucial biological process that shapes evolutionary change within and between species. At the same time, accurate estimates of recombination rates are essential for correct inferences of selection and demographic events. Importantly, recombination rate is also a fast evolving trait with closely related species showing clear differences in the distribution of crossovers (CO) along chromosomes. Population genetics models that incorporate patterns of Linkage Disequilibrium (LD) can be used to survey recombination variability across the whole genome based on (now cheap) genomic sequences. In this study, we use the most recent and accurate population genetic method, LDhelmet, to estimate recombination rates in several *Drosophila melanogaster* populations and compare them to a high resolution recombination map obtained experimentally. Our analysis suggest there have been recent and significant changes in the recombination rate landscape across the *D. melanogaster* genome. We show that not all populations have acquired differences in their recombination landscape at the same rate and scale. We also show the results of a study designed to test whether population differences in recombination rates have played a detectable role explaining differences in nucleotide diversity among populations. .

692A

Rates of crossing over gradually increase over time in *Drosophila melanogaster* females. Chad Hunter, Matthew Robinson, Nadia Singh. Program in Genetics, Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695.

Meiotic recombination is an essential biological process that ensures proper segregation of homologous chromosomes in many organisms. Interestingly, rates of recombination vary greatly both due to genetic and environmental factors. Early studies in Drosophila revealed that rates of recombination change with advancing maternal age but the direction and magnitude of change still remain debated. Here, exploiting a two-step crossing scheme and homozygous, visible markers, we measured rates of crossing over in a 33 cM interval on the X and in 20.4 cM interval on the 3R chromosome over a 21-day period. We used five inbred wild-type strains in this experiment. Our results indicate that rates of crossing-over increase as a function of maternal age across all genotypes. However, we also find that the relationship between maternal age and recombination frequency is mediated in part by genetic background. For instance, while one line showed a 1.5-fold increase in crossover frequency over 21 days, another line showed a much more modest increase of 1.15-fold. Moreover, the magnitude of the increase associated with increasing age varies between the two intervals surveyed. This work highlights the complex nature of recombination rate variation in Drosophila as a function of maternal age, genetic background, and their interaction.

693B

ConTExt: A Strategy for Studying the Structure and Population Dynamics of Transposable Element and Repeat Content. Michael P McGurk, Daniel Barbash. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Transposable elements (TEs) are mobile elements that can replicate within a host genome and often comprise a large proportion of eukaryotic DNA. The selfish gene theory casts TEs as genetic parasites that, despite deleterious impacts on the host, have colonized and evolved with genomes throughout life's history. Yet, while this is likely the major force driving TE content, the overall evolution is quite complex; even the within-species dynamics of TE abundance remain unclear. Further, TEs are mutagens, potent mediators of genome rearrangements, especially within the repeat-rich heterochromatin. The most complete studies of TE content have been restricted to the genomes of individuals, while those carried out at a population-level typically focus only on TEs interspersed in unique sequence, ignoring those in repeat-rich regions. Here we present ConTExt, a strategy to bridge this gap. Utilizing paired-end reads, our pipeline can discover the junctions corresponding to TE insertions and other repeats in unique sequence, nested in other repeats, or arranged in tandem arrays, as well as the deletions and inversions that inactivate elements. The inference of junctions is complemented by a novel approach to visualizing paired-end data which presents the alignments as 2-dimensional images, allowing for easy manual analysis and validation; an image processing algorithm is used to automatically identify junctions. Our approach readily identifies the known *Bari* tandem array, TE insertions into the highly repeated ribosomal genes, and even stocks in which all copies of an element are inactive. Applying this to the hundreds of datasets available for diverse populations and species of *Drosophila*, ConTExt will not only capture between-population differences in TE abundance, but also the proportions of inactive elements and the complex structure of heterochromatin, providing a comprehensive picture of how the content of TEs and other repeats varies between populations.

694C

Sex determination and gene expression in the house fly, *Musca domestica*. Richard Meisel[1], Jeffrey Scott[2], Andrew Clark[2]. 1) University of Houston; 2) Cornell University.

Sex determination (SD) pathways evolve very fast. Theory predicts that the evolutionary turnover at the top of SD pathways could be driven by natural selection, inter-sexual conflict, or sex-ratio selection. The house fly, *Musca domestica*, is an ideal model to study the evolution of SD. Canonical house fly females have two X chromosomes (XX) and males are XY, with a dominant male-determining locus, M, on the Y chromosome. M can also be found on the autosomes and the X chromosome in natural populations, but XY males are believed to be the ancestral state. M-bearing autosomes form stable geographic clines, M-bearing chromosomes have different fitness effects in laboratory assays, and M alleles differentially affect splicing of downstream SD genes, suggesting that the M polymorphism has phenotypic effects that could be acted upon by selection. To test that hypothesis, we used RNA-seq to measure gene expression in head and testis, and we compared expression profiles between males carrying Y-linked M (Y^M) and males with M on the third chromosome (III^M). We detect more differential expression between Y^M and III^M males in testis than head, and an excess of the differentially expressed genes also have male-biased expression. These results suggest that reproductive and male-specific phenotypes are disproportionately affected by the M polymorphism, and we identified candidate genes whose differential expression may contribute to phenotypic differences between Y^M and III^M males. In addition, genes that are up-regulated in III^M males are more likely to have male-biased expression than genes up-regulated in Y^M males. The third chromosome harbors ~20% of house fly genes, whereas the Y chromosome has no known genes other than M and is not necessary for viability or fertility. Our results suggest that the male-limited transmission of the gene-rich III^M chromosome favored the accumulation of male-beneficial alleles, whereas the gene-poor Y chromosome does not realize such a benefit. We hypothesize that the fixation of male-beneficial alleles on III^M promoted the invasion of III^M in natural populations because of male-specific or sexually antagonistic selection.

695A

Population genetics of *Drosophila eremophila* in Mexico. Alejandra J. Vieyra-Ramirez, Therese A. Markow. LANGEBIO - CINVESTAV, Irapuato, Guanajuato, Mexico.

Most species from the *Drosophila repleta* group are associated with cacti in arid or semiarid regions in the New World. Their unusual ecology makes them good model systems to study the roles of abiotic and biotic factors in ecological specialization.
The *eremophila* complex of the *repleta* group is composed of *Drosophila eremophila*, *D. mettleri* and *D. micromettleri*. Of the three, *D. mettleri* is known to breed in soil soaked by the juice of necrotic cacti and its ecology and genetics have been the best studied. *Drosophila eremophila* is the ancestral species and is endemic to Mexico. Its biology, distribution, and population structure in

Mexico remain largely unknown. We provide a preliminary analysis, using mitochondrial cytochrome c oxidase subunits I and 2 (COI and CO2) genes, of population relationships for widely distributed populations in Mexico. No population structure was detected among *D. eremophila* samples from Baja California, Tamaulipas, San Luis Potosi, Guanajuato, Queretaro and Hidalgo, indicating that the species exists as a large panmictic population.

696B

Mating system of an invasive species, *Zaprionus tuberculatus* malloch, 1932, (diptera:Drosophilidae). Ergi D özsoy[1*], Bahar Patlar[1,2]. 1) Hacettepe University, Department of Biology, 06800 beytepe campus, Ankara, Turkey; 2) Bielefeld University, Department of Evolutionary Biology, 33615, Bielefeld, Germany.

The invasive drosophilid species *Zaprionus tuberculatus* was first captured in 2011 in southeastern coast of Mediterranean region, Adana, Turkey. It is supposed that the first access of the species into the region must have been a recent event and it has become established and increased its number in the region in a relatively short time. Accordingly, the importance of reproductive system for the invasion biology prompted us to characterize some mating components of *Z. tuberculatus* sampled from the region. Within this framework we determined the age at sexual maturity in both sexes, average egg number laid by a female after copulation, duration of the copulation and the testis length. In performing these, we used isofemale lines constructed from two altitudinal samples of the region to reveal how reproduction in turn affect the organization of observed variation within and among populations. We found that both sexes become mature on the third day after eclosion in both samples. Average daily egg number laid by females showed different patterns after copulation but no significant differences were detected between the samples. Nevertheless, the means of copulation duration and testis length were significantly different between the samples. We suggest, in light of the uniformity of the different altitudinal samples with respect to the age at sexual maturity and average daily egg number, that these traits may be evolutionarily conserved in this invasive species, though the copulation duration and testis length may not. .

697C

Differential Gene Expression Patterns Of Chromosomal Inversion Karyotypes Provide Insight Into The Evolutionary Mechanisms Maintaining Third Chromosome Gene Arrangements of *Drosophila pseudoobscura*. Zach Fuller[1], Gwilym Haynes[1], Shannon Duggan[2], Dianhuiz Zhu[2], Stephen Richards[2], Stephen Schaeffer[1]. 1) Biology, Penn State, University Park, PA; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Drosophila pseudoobscura provide an ideal system to test hypotheses regarding the maintenance of chromosomal inversions in natural populations, as the third chromosome is polymorphic for over 30 different gene arrangements. The arrangements were generated through a series of overlapping paracentric inversions. Arrangement frequencies are clinally distributed in nature, despite the predicted homogenizing effects of extensive migration. Previous genome sequencing detected a number of arrangement-specific, fixed nucleotide sites across the inverted regions where recombination is suppressed between chromosome heterokaryotypes. It is hypothesized that suppressed recombination may hold together sets of locally adapted alleles and alter patters of gene expression between arrangements. In several geographic regions multiple arrangements exist at high frequency, providing the opportunity for chromosome heterokaryotypes to form. Thus, it is also hypothesized that heterosis may act to maintain arrangement frequencies in regions where inversion heterozygotes are common. To test these hypotheses, the transcriptome of first instar larvae and unmated adult males and females was sequenced for seven common gene arrangements made homozygous for the third chromosome, and in four common inversion heterozygotes. Differences in gene expression was examined between arrangements and life stages. The frequency of differentially expressed genes was significantly higher within the inverted segments of the chromosome. These genes are enriched for processes involved in detoxification and sensory perception pathways. Gene expression patterns were also significantly different between third chromosome heterokaryotypes and homozygous individuals, with evidence for prevalent, semi-dominant interactions of gene copies derived from different chromosome arrangements. These results provide insight into the evolutionary forces responsible for the maintenance of gene arrangements of *D. pseudoobscura*.

698A

Genomic evidence for non-neutral evolution of *In(3R)Payne*, a major clinal chromosomal inversion in *Drosophila melanogaster*. Martin Kapun[1], Jérome Goudet[1], Paul Schmidt[2], Thomas Flatt[1]. 1) Department of Ecology and Evolution, University of Lausanne, Switzerland; 2) Department of Biology, University of Pennsylvania, Philadelphia, USA.

Several chromosomal inversion polymorphisms in *D. melanogaster* exhibit steep and persistent latitudinal clines across different continents, suggesting that they may be shaped and maintained by spatially varying selection. However, the adaptive nature of chromosomal inversions is still controversial, and how chromosomal inversions affect genome-wide patterns of genetic variation in natural populations remains poorly understood. Here we address this fundamental issue by analyzing Pool-Seq data from 10 populations along the North American east coast (generated by the NESCent *Real Time Evolution*Consortium), complemented by data from additional populations in Europe and Australia. We used inversion-specific marker SNPs to estimate inversion frequencies and found clear evidence for the temporal stability of inversion clines and for strong correlations with climatic factors, especially for *In(3R)Payne*. Consistent with the action of clinal selection, Q_{ST}-F_{ST} analysis of the North American cline revealed that *In(3R)Payne* is maintained by non-neutral evolution. By applying SNP-wise linear regression we also detected alleles strongly correlated with *In(3R)Payne*; all associated SNPs were located within or in close proximity to the inversion. Multiple candidate SNPs clustered within the inversion body away from the breakpoints, a pattern inconsistent with neutral expectations. Intriguingly, and in agreement with these observations, these candidates are also strongly differentiated between the endpoints of the evolutionarily independent but

parallel cline along the Australian east coast. Together, our results provide compelling genomic evidence that *In(3R)Payne* is shaped and maintained by spatially varying selection across multiple continents.

699B

Widespread transposon landscape diversity amongst *Drosophila* cell cultures and fly strains. Reazur Rahman, Yuliya Sytnikova, **Nelson Lau**. Biology, Brandeis University, Waltham, MA.

Because transposons are powerful mutagens that lurk in all animal genomes, the Piwi pathway naturally suppresses transposons from mobilizing in the germline. Despite the robustness of the Piwi pathway in transposon silencing to enable fertility, transposons have remained a sizeable ~10% of the *Drosophila* euchromatic reference genome. In the *Drosophila* follicle cell lines, called OSCs and OSS cells, we detected a substantial diversity in transposons landscapes through our bioinformatics platform called TIDAL (Transposon Insertion and Deletion AnaLyzer). These transposon landscapes resulted in distinct transcriptomes within OSCs and OSS cell lines because genes with new transposon insertions nearby were repressed by the spreading of PIWI-directed chromatin silencing; whereas other transposons were stimulating the expression of novel long non-coding RNAs (lncRNAs). We extended TIDAL to the analysis of sequenced genomes from Drosophila cell culture lines, fly lab strains and wild type fly strains from the *Drosophila* Genetic Reference Panel and the *Drosophila* Population Genomics Resource. We find that transposon landscape diversity is quite widespread when comparing to the reference genome strain *y[1]; cn[1] bw[1] sp[1]*. Transposon InDels ranged from thousands amongst cell cultures to several hundreds of transposon InDels amongst wild-type and lab fly strains. Interestingly, the comparison of transposon depletion patterns versus insertions strongly supports retrotransposition as the dominant mode of global transposon mobilization. The transposon landscapes generated by TIDAL from the sequenced genomes of fly cells and strains will be useful to the fly research community for interpreting gene expression profiles.

700C

The Molecular Basis of Segregation Distortion in *Drosophila pseudoobscura*. Randee E. Young[1], Zachary L. Fuller[2], Stephen W. Schaeffer[2], Nitin Phadnis[1]. 1) Department of Biology, University of Utah, Salt Lake City, UT; 2) Department of Biology, Pennsylvania State University, University Park, PA.

According to Mendel's Laws of Inheritance a gene or chromosome is transmitted from parent to progeny in a 1:1 ratio such that each copy has an equal chance of being transmitted to the offspring. Segregation distorters are selfish genetic elements that violate Mendel's Laws of Inheritance. These cheating chromosomes act by over representing themselves in the mature gamete pool, and thus propel themselves through populations even though this often comes at a fitness cost to the host. Segregation distorters are ubiquitous in nature and have long been recognized as a powerful evolutionary force. Yet very few segregation distortion genes have been identified to date. We are interested in understanding the molecular basis of segregation distortion in the classic *Sex-Ratio (SR)* system in *Drosophila pseudoobscura*. *SR* is an X-linked distorter system, such that males carrying the *SR* chromosome produce only X-bearing sperm resulting in nearly 100% female progeny. The *SR* chromosome differs from the Standard X chromosome (ST) by three non-overlapping inversions. The segregation distorter genes reside within these inversions, frustrating traditional genetic approaches to identify them. We have performed next generation sequencing on wild caught *D. pseudoobscura SR* males and ST males to characterize the genetic differences between these chromosomes. We have identified the precise breakpoints of two of the three chromosomal inversions, and made a comprehensive list of potential gene candidates based on fixed non-synonymous changes in the *SR* chromosome. Our results offer a glimpse into the evolutionary history of this enigmatic selfish genetic system.

701A

***Dorsocross* provides key to morphological divergence of extraembryonic development. F. Caroti**, S. Lemke. Dept. of Molecular Developmental Biology and Physiology, Centre for Organismal Studies (COS), Heidelberg, Baden Württemberg, Germany.

Gastrulation defines a critical step in the early embryonic development and is comparable among Diptera ("true" flies). During gastrulation, flies form a specialized extraembryonic tissue that eventually seals the embryo dorsally. In most flies and insects, this extraembryonic tissue consists of two distinct epithelia, the amnion and the serosa, which develops by the extension of an epithelial fold (so-called amnioserosal fold). In *Drosophila melanogaster*, the extension of this amnioserosal fold is strongly reduced and only a single epithelium is formed, the amnioserosa. Although this morphological difference, extraembryonic tissue in all flies is specified by the homeobox gene *zerknüllt* (*zen*). We are interested in the cellular and genetic mechanisms that changed during evolution downstream of *zen* and gave rise to the single epithelium observed in *D. melanogaster*. One of the previously identified target genes of *zen* is *dorsocross* (*doc*), a gene known to be fundamental for fold formation in wing imaginal discs in *D. melanogaster*. Combining functional analyses and high resolution *in vivo* imaging, we have studied the expression pattern and function of *dorsocross* orthologues in embryos of the scuttle fly *M. abdita*, which develops an amnion and a serosa. Our results suggest that *dorsocross* and its downstream targets have acted as primary switches in the control and divergence of extraembryonic development during the course of fly evolution.

702B

Exploring the origin of insect wings through functional analysis of *vestigial* in various insect species. C. M. Clark-Hachtel[1], D. M. Linz[1], X. Bellés[2], E. Buschbeck[3], Y. Tomoyasu[1]. 1) Department of Biology, Miami University, Oxford, OH; 2) Institut de Biologia Evolutiva, Barcelona, Spain; 3) Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio.

Despite accumulating efforts to unveil the origin of insect wings, it remains one of the principal mysteries in evolution. Currently, there are two prominent models regarding insect wing origin: one connecting the origin to the paranotal lobe and the other to the

proximodorsal leg branch (exite). However, neither hypothesis has been able to surpass the other. To approach this conundrum, we focused our analysis on *vestigial* (*vg*), a critical wing gene initially identified in *Drosophila*. Our investigation in the *Tribolium* beetle has revealed that, despite the well-accepted view of *vg* as an essential wing gene, there are two groups of *vg*-dependent tissues in the "wingless" first thoracic segment (T1)[1]. Further investigation has revealed that these tissues are wing serial homologs in a wingless segment. Intriguingly, these two T1 wing homologs may actually be homologous to the two proposed wing origins (paranotal lobes and exite-bearing proximal leg segments). Therefore, our findings suggest that the *vg*-dependent tissues in*Trobolium* T1 could be wing serial homologs present in a more ancestral state, thus providing compelling functional evidence for the dual origin of insect wings. We are currently testing this model by (i) analyzing the nature of the *Tribolium* T1 wing serial homologs via RNAseq and (ii) evaluating the presence of the T1 wing homologs in another beetle (a diving beetle, *Thermonectus*) and a hemimetabolous insect (a cockroach, *Blattella*) to determine the lineage specific nature of the *vg*-dependent tissues in the wingless segments. 1. Clark-Hachtel, C. M., Linz, D. M. & Tomoyasu, Y. Insights into insect wing origin provided by functional analysis of *vestigial* in the red flour beetle, *Tribolium castaneum*. *PNAS* **110**, 16951-16956, (2013).

703C

Dissecting the genetic basis and evolution of form using haploid wasps. Lorna Cohen, Jeremy A Lynch. University of Illinois at Chicago, Chicago, IL.

Identifying and characterizing the molecular basis responsible for differences in form among species is one of the major goals of Evo-Devo. The *Nasonia* genus is an emerging model clade for such studies. Of the four species within the *Nasonia* genus, we are interested in *N. vitripennis and N. giraulti* for morphology studies. These species can be made interfertile in the lab by curing Wolbachia infections. *N. vitripennis* has a well sequenced and annotated genome. Additional sequencing is underway to make the genomic tools in *N. giraulti* comparable to those of *N. vitripennis*. *N. giraulti* is of particular interest as the head shape of the males is distinct from the other species. Further, *Nasonia* have haplodiploid genetics; fertilized eggs give rise to females and unfertilized eggs give rise to haploid males. This characteristic is particularly useful in the study of interspecies differences in male traits, since the haploid male progeny of hybrid females can be screened directly in the F2 generation. In addition to the interspecies shape differences, the haploid genetics of *Nasonia* is a particular advantage in mapping epistatic interactions among multiple loci. Hybrid crosses reveal negative epistatic phenotypes that are not present in either of the wild type species. We are testing the applicability of Multiplex Shotgun Genotyping, which should allow high resolution and high throughput genotyping of hybrid males. Lastly, we are characterizing the developmental basis of head shape in these wasps by examination and comparison of eye-antennal discs between species.

704A

Giant causes hybrid inviability in *Drosophila*. Jackie Gavin-Smyth[1], Marty Kreitman[1], John Reinitz[1], Daniel Matute[2]. 1) Dept Ecology and Evolution, University of Chicago, Chicago, IL; 2) Biology Department, University of North Carolina, Chapel Hill, North Carolina.

Despite millennia of evolutionary divergence time, many genes responsible for early axial patterning of the embryo are highly conserved on the levels of coding sequence and spatio-temporal expression. This is especially true within the genus *Drosophila*, as even very distantly related flies share both adult morphology and early embryonic gene expression patterns. This gross similarity has led to the presumption that such genes are functionally constrained by strong selective pressures. We have begun testing the functional conservation of orthologous early developmental patterning genes and their *cis*regulation using an interspecific hybridization scheme: crosses between females of the genetic model organism *D. melanogaster*, and males of an island endemic, *D. santomea,* species whose most recent common ancestors were 12-15 MYA. Although these species are highly diverged, this cross produces sterile adult females, and males that are rendered inviable during embryogenesis. These male hybrid embryos manifest a striking abdominal ablation phenotype. Using both deficiency and duplication mapping, we have traced the genetic determinant of this sex specific abdominal ablation phenotype to the*melanogaster X*-linked gap gene *giant*. Quantitative imaging and detailed analysis of segmentation and cell fate specification throughout embryogenesis reveals that the *D. melanogaster giant* allele has complex and aberrant behavior in the *D.melanogaster/D.santomea* hybrid embryo context. We determined that it is the functional divergence between *D.melanogaster* and *D.santomea giant* alleles that gives rise to the ablation phenotype. Taken together, these results reveal a broader diversity of protein functions and *cis* regulatory dynamics than was previously appreciated.

705B

Conserved gene expression in the absence of sequence conservation: Uncovering a molecular mechanism by theoretical and experimental analysis of *even-skipped* expression from highly diverged sequences. Ah-Ram Kim[1,2], Pengyao Jiang[2], John Reinitz[2]. 1) Computer Science & Artificial Intelligence Laboratory, MIT, Cambridge, MA; 2) Department of Ecology and Evolution, Chicago Center for Systems Biology, University of Chicago, Chicago, IL.

The expression of metazoan genes is governed by enhancers: regulatory DNA sequences containing transcription factor binding sites. The concentration and identity of transcription factors on enhancers are the primary determinant of target gene expression levels. While the enhancer activity can be highly sensitive to minute sequence changes that disrupt critical binding sites, orthologous enhancers can drive nearly identical expression patterns despite a complete absence of sequence homology. This conservation of spatiotemporal enhancer activity is widely observed in various species from Nematodes to Vertebrates. To better understand this phenomenon, and gene regulation as a whole, we wished to determine the molecular mechanism underlying conservation of gene expression without the conservation of sequence. Here we show how this problem can be solved by combining a computational model of *cis*-regulatory logic and genetic manipulation. We elucidate the molecular mechanisms of conserved gene expression driven by *even*-

skipped stripe 2 enhancers of the families Drosophilae and Sepsidae. While stripe 2 expression is driven by synergistic activation of Bicoid and Hunchback in the *Drosophila*enhancers, the divergent *T. putris* enhancer relies on a completely different *trans*-acting regulator, Caudal, to activate expression in the *D. melanogaster* embryos. The discovery that the posterior gap gene Caudal can drive stripe 2 expression, instead of the anterior pattern regulator Bicoid, illustrates the remarkable flexibility of metazoan enhancers in using both DNA sequence and a *trans*-acting network to maintain an essential function in the course of *cis*-regulatory evolution.

706C

A re-evaluation of *bicoid* in Diptera. Jeff Klomp, Urs Schmidt-Ott. Organismal Biology and Anatomy, University of Chicago, Chicago, IL.

Formation of the primary body axis is a fundamental step in the embryonic development of the fruit fly, Drosophila. Head-to-tail polarity of the primary axis is established by anterior localization of mRNA from the homeobox transcription factor gene bicoid. Subsequent translation of the bicoid mRNA results in a protein gradient toward the posterior. Drosophila embryos deficient for bicoid fail to develop proper head-to-tail polarity and instead form embryos with a second abdomen in place of the head. Even though bicoid performs a crucial role in development of Drosophila, it is absent from most other flies. We sought to reevaluate the prevalence of the bicoid gene using available genomic and transcriptomic data. Using BLAST searches we confirmed the absence of bicoid in lower flies (non-cyclorrhaphans) and its presence in several lineages of higher flies (cyclorrhaphans). However, genomic and transcriptomic data do not support the presence of bicoid in all cyclorrhaphan groups, suggesting the possibility of repeated evolution of anterior determinant genes in the course of the dipteran radiation.

707A

Genetic basis of pigmentation divergence between closely related *Drosophila* species: interactions between *tan* and *yellow*. Abigail M. Lamb[1], Alisha V. John[1], Arielle M. Cooley[2], Lisa L. Sramkoski[1], Patricia J. Wittkopp[1,2]. 1) Department of Molecular, Cellular, & Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

An increasing body of evidence supports the hypothesis that divergence in *cis*-regulatory DNA sequence contributes to phenotypic diversity. However, questions remain about the precise mechanisms by which changes in regulatory sequence lead to novel phenotypes. Divergent expression of two genes, *tan* and *ebony,* has been shown to contribute to the derived light yellow body color of *Drosophila novamexicana,* a unique trait among members of the darkly pigmented *virilis* subgroup. Using a transgenic approach, we aim to pinpoint causative non-coding changes in *D. novamexicana tan* that lead to changes in pigmentation relative to its melanic sister species, *D. americana*. To identify these sites, we expressed *D. novamexicana tan, D. americana tan*, and chimeric transgenes composed of sequence from both species in *D. melanogaster tan* mutant flies and compared pigmentation among transgenic lines. We found that substitution of a ~1.2kb portion of *D. novamexicana* intron 1 into *D. americanatan* lightened pigmentation to a level indistinguishable from flies expressing *D. novamexicana tan*. Interestingly, this effect was not detectable in *yellow* mutant flies, suggesting that synergistic activities of *tan*and *yellow* may be necessary for detection of subtle differences in pigmentation in this system. The identified candidate region contains only four SNPs that are derived in *D. novamexicana*. We are currently using CRISPR/Cas9 genome editing to interrogate the phenotypic effects of these SNPs.

708B

Recapitulating the evolutionary origin of ventral furrow formation. Silvia Urbansky, Paula Gonzalez, **Steffen Lemke**. Centre for Organismal Studies, Universitaet Heidelberg, Heidelberg, Germany.

During gastrulation of *Drosophila melanogaster*, the presumptive mesoderm involutes as a continuous epithelial sheet along the ventral midline and forms a characteristic ventral furrow. At the cellular level, ventral furrow formation is initiated in mesodermal cells by coordinated apical constriction of actomyosin. This coordinated constriction of actomyosin is controlled by the transcription factors Twist and Snail, which activate GPCR-signaling via Folded Gastrulation (Fog) and lead to cortical localization of RhoGEF2 via Concertina and T48. Despite its critical role in gastrulation of *D.melanogaster*, recent analyses as well as classical literature suggest that mesoderm internalization through epithelial involution constitutes an evolutionary novelty. To identify the genetic basis for this morphogenetic novelty, we quantified gastrulation dynamics in the midge *Chironomus riparius*, which shares its last common ancestor with *D.melanogaster* 250 million years ago, and in which mesodermal cells ingress individually and in a stochastic manner. By systematically characterizing the genetic and cytoskeletal regulation of mesoderm formation in *C.riparius* we made two key observations: (1) *fog* and *T48* are differentially expressed between *D.melanogaster* and *C.riparius*, and (2) the induction of *D.melanogaster*-like expression of both genes in *C.riparius* blastoderm embryos changes mesodermal cell behavior from stochastic and individual ingression towards coordinated tissue involution, and thus a more *D.melanogaster*-like behavior. Our results suggest that experimental elevation of *fog* and *T48* expression is sufficient to coordinate mesoderm cell behavior, and thus artificially recapitulates the evolutionary origin of ventral furrow formation. We propose that gastrulation in *C.riparius* provides an example of a poised evolutionary system, in which gradual changes within existing regulatory networks can invoke fundamental, non-gradual transformation in mode and outcome of morphogenesis.

709C

Exploring the molecular basis of insect wing evolution: a transcriptomic approach. David Linz, Yoshinori Tomoyasu. Zoology Department, Miami University, Oxford, OH.

We are studying the gene regulatory network of wing development in *Tribolium* (the red flour beetle) and comparing it to that of the fruit fly, *Drosophila,* to understand the molecular basis of morphological evolution. The wings of these two insects have become vastly different over evolutionary time. The fly has typical flight wings on the second thoracic segment (T2), but has intensively modified wings (halteres) on T3. In contrast, the beetle has a pair of hardened protective structures (elytra) on T2, and uses the T3 hindwings for flight. We have been analyzing the function of potential "wing genes" (selected from previous *Drosophila* studies) in *Tribolium* wing development: *i.e* a candidate gene approach. However, as these studies have progressed, the choices of candidate genes have become increasingly limited and also created a fly-biased view of insect wing evolution. To gain a less biased view of insect wing evolution, we have started exploring genes that could be uniquely important for the beetle wing development, and thus not present in *Drosophila* wing development. We first examined a class of developmental genes (toolkit genes) that are known to be important for embryonic segmentation in *Drosophila*. These toolkit genes tend to show a high degree of pleiotropy, therefore increasing the likelihood of finding novel wing genes in the beetle. Despite this, we found no definitive examples of beetle wing genes whose orthologs in fly are not important for wing development. To obtain further insight into the molecular basis of insect wing evolution, we adopted a *bona fide* non-candidate gene approach, RNA sequencing. We are currently analyzing the *Tribolium* wing transcriptome (both elytron and hindwing), and comparing them to those of other insects, which will allow us to identify both conserved wing genes as well as wing genes uniquely co-opted in the beetle lineage. We will then analyze the function of a subset of genes uniquely expressed in *Tribolium* wings by performing large scale RNAi in *Tribolium*. This work will provide the first comprehensive examination of genes present in the beetle wings and will thus provide further insights into the molecular mechanisms driving the evolution of morphology.

710A

The shared genetic architecture of sleep and reproductive fitness in *Drosophila melanogaster*. A. Lobell, R. Kaspari, S. Harbison. Laboratory of Systems Genetics, National Heart, Lung & Blood Institute, Bethesda, MD.

Sleep is universal among animals. While this conservation suggests important fitness benefits, the relationship between sleep and evolutionary fitness is unknown. One possibility is that sleep shares genetic architecture with other fitness traits. Here, we investigate the shared genetic architecture of sleep and reproductive fitness in *Drosophila melanogaster*. We focus on the ovarioles, which produce oocytes and affect fitness by limiting maximum female fecundity. We measured ovariole number in 202 lines of the *Drosophila* Genetic Reference Panel (DGRP) and quantified the asymmetry between ovaries. We also calculated the ovariole number coefficient of environmental variation (CV_E) to determine the degree to which environmental sensitivity is influenced by underlying genotype. Ovariole number and its CV_E show significant phenotypic variation among lines ($P < 0.0001$) with high broad-sense heritability (ovariole number $H^2 = 0.59$; CV_E $H^2 = 0.23$). Ovariole asymmetry also exhibits significant variation, but with lower heritability ($H^2 = 0.05$). We used genome-wide association studies to identify genetic variants underlying these traits. We identified 101, 1,911 and 172 variants significantly associated with ovariole number, ovariole number CV_E and ovariole asymmetry, respectively. These map to 627 genes, which are enriched for biological processes including regulation of developmental processes ($P = 1.03 \times 10^{-5}$), cell adhesion ($P = 3.14 \times 10^{-4}$) and organ morphogenesis ($P = 0.00025$). Further, we identified genes biologically plausible for affecting ovarian morphology including *bru-3*, *Mdr49*, *zfh1*,*bab1* and *fru*. By comparing these results to 8,197 single-nucleotide polymorphisms previously associated with 14 sleep traits, we identified 30 common genes. We used *P*-element insertion- and RNAi expression knockdown- lines as a proxy to verify these genes' dual effects. Preliminary results indicate significant overlap in genetic architecture; genes including *bru-3, kirre, bin-3 and CG42388* were found to have pleiotropic effects on ovariole number and sleep. Such pleiotropic effects may explain why sleep has been so strongly conserved over evolutionary time.

711B

The natural symbiosis of Drosophila and yeasts in a vineyard. Allison Quan[1], Kelly Schiabor[1], Michael Eisen[1,2]. 1) Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, Berkeley, CA.

Though often studied independently, *Drosophila* and yeast participate in a symbiotic relationship in nature. Little is known about this ecological association, its essential chemistry, and its influence on the evolution of both organisms. To determine the specificity of this relationship in nature, we collected *Drosophila* and fungi from an organic, Northern California vineyard over an entire harvest season. We determined *Drosophila* species follow a specific spatial distribution in the vineyard: *D. melanogaster* was collected solely on fermenters in the winery while *D. simulans* was found exclusively inside grape clusters in the vineyard. Temporally, Drosophilids only appeared in the vineyard once grapes had matured, as fungal diversity was peaking. We found that the fungal community structure present on grapes, while not correlated with available nutrient levels, is distinct between grape varietals. Of all the 41 yeast species found on the grapes, less than 5% were vectored by *Drosophila*, which carried two unique yeast species: *Issatchenkia hanoiensis* and *Candida zemplinina*. We are currently using behavioral assays to characterize species-specific *Drosophila* responses towards natural yeast species collected in the vineyard and to identify the fungal-produced volatile compounds that mediate these fly responses.

712C

Next-generation approaches to understanding the evolution of insect germline. Honghu Quan, Jeremy Lynch. University of Illinois at Chicago, Chicago, IL.

Germline cells are unique as they can produce gametes and regenerate themselves. Among animals, germ cells can be specified by either maternally inherited determinants or by inductive signals. Although maternal determination mode is found in most model

organisms (fruit fly, zebrafish, frog and nematode), it is actually the less prevalent mode of germline specification. Whereas the zygotic induction may be the ancestral model of germline determination for most animals.
Among the invertebrates, the only arthropod in which the germ line has been studied in detail is *Drosophila melanogaster*. However, this mechanism of germ cell specification is not widespread among, or representative of, all arthropods. *Nasonia*, like *Drosophila,* using the maternal inheritance mode to establish its germline, represents an earlier lineage than *Drosophila* does in Holometabla, which might give the hint to explain the origin of this mode. The assembly of *Nasonia's* germ plasm is dependent on a regulatory network that is very similar to that of *Drosophila*, and occurs in the same context of polytrophic ovaries. Despite the overall similarity between *Nasonia* and *Drosophila* oogenesis and embryogenesis, the *Nasonia* germ plasm and pole cells have some distinct features from their *Drosophila*counterparts. To understand the conserved and divergent features between *Nasonia* and *Drosophila* from the molecular level, one of the most abundant and important components of germ plasm, mRNA, was characterized by RNAseq. The results confirmed that certain genes, such as *oskar, nasos*, etc., are conserved between the two species, and showed some unknown genes that are still under investigations. .

713A

Convergent evolution of female-limited color dimorphism in *Drosophila*. Amir Yassin[1], Héloïse Bastide[1], Justin Lack[1], Jean David[2], John Pool[1]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Laboratoire Evolution, Génomes et Spéciation, CNRS, Gif-sur-Yvette, France.

Drosophila melanogaster got its name from the characteristic dark pigmentation at the tip of the male abdomen. However, this flagrant sexual dimorphism, which has attracted the attention of developmental geneticists for a long time, is a derived state within the genus *Drosophila*. In the *montium* species group, which is closely-related to *melanogaster*, a fascinating yet largely unexplored pattern of sexual dimorphism has evolved. In more than 20 species of this group, the color dimorphism is restricted only to females, with one morph resembling males, which are monomorphically light or dark depending on the species. By combining intra- and inter-specific introgression mapping and comparative genomics we identified a ~275 kb interval on chromosome 2R to be associated with female-limited color dimorphism (FLCD) in *D. leontia*, a species from the *kikkawai* complex. Excess FLCD-associated shared SNPs in *D. leontia* and *D. kikkawai* was found in this region around the long transcription factor gene *pdm3*, which is known to affect pigmentation differently between the sexes in *D. melanogaster*. Although the dark alleles are probably ancestral, we provide support for the recent introgression of the light alleles from *D. kikkawai* into *D. leontia*. However, no evidence for shared genetic basis of FLCD with other dimorphic *montium* species beyond the *kikkawai* complex was found. Since FLCD has multiple origins and segregates in natural populations, it offers a unique opportunity for the study of the evolution of *Drosophila* sexual dimorphism in action.

714B

Scanning Electron Microscopy of immature stages of *Drosophila willistoni* group. Rebeca Zanini[1,2], Maríndia Deprá[1,2], Vera Valente[1,2]. 1) Programa de Pós-Graduação em Biologia Animal - UFRGS, Porto Alegre, Brazil; 2) Laboratório de Drosophila, Departamento de Genética - UFRGS, Porto Alegre, Brazil.

Drosophilidae family presents a variety of color and body types that are diagnosis for subfamilies, genus and even species. Immature stages of Drosophilidae have variable characters in the genus and subgenus, such as egg filaments, larvae and puparia spiracles. Some differences in these stages only could be observed under Scanning Electron Microscopy. *Drosophila willistoni* group is mostly Neotropical and currently includes 23 species divided in three subgroups: *alagitans, bocainensis* and *willistoni.* The *willistoni* subgroup is cryptic and is composed of five species and a superspecies *D. paulistorum*, which emcompasses six semispecies. The objective of this study was characterize and compare the morphology of the eggs, larvae and pupae of four species of *bocainensis* subgroup and four species and the *D. paulistorum* semispecies of the *D. willistoni* subgroup aiming to find differences between them that could be used for phylogenetic purposes. Eggs, third instar larvae and pupae were fixed with glutaraldehyde and washed with crescent concentrations of acetone. The samples were metalized with gold and visualized in a Scanning Electron Microscope.The general morphology of the eggs is similar; the eggs of all examinated species presented two respiratory filaments, with minor differences regarding size and surface ultrastructure, which varies from solid to porous, but the shape is unique for most of the analyzed species. We also observed differences in operculum and micropyle, but these were not species specific. The larvae and pupae of the *bocainensis* subgroup are larger than those of*willistoni* subgroup. Some characters of the pre-adult stages could be useful for species identification and evolutionary studies. .

715C

Effects of space missions on stability of Drosophila estimated by fluctuating asymmetry. Denis V. Anisiforov, Alex M. Kulikov, Oleg E. Lazebny. Russian Academy of Sciences, Koltzov INSTITUTE of DEVELOPMENTAL BIOLOGY, Moscow, Russian Federation.

Differences in the wing size and shape in *Drosophila melanogaster* flies visiting for two weeks the International space station comparing to their Earth controls were studied. The so-called geometric morphometric approach was used for estimation of the wing shape. The aims of the study were:

- to estimate the relationship of fluctuating asymmetry (FA) on the disadvantageous factors of space mission;
- to estimate the expression of FA in females and males;
- to estimate the difference in FA of inbred and outbred *Drosophila* strains.

The increasing of FA indices in space flies was revealed. Females were more resistant to adverse factors of the space flight comparing to males. Outbred flies were characterized by lower FA values comparing to inbred fellows. Unfavorable effects of space missions as stressful factors affecting ontogeny in Drosophila flies are discussed on the base of the results obtained.

716A

Sexual traits as a measure of evolutionary divergence in *Drosophila nasuta* subgroup members. Shruthi Balachandra[1], Kristipati Ravi Ram[2], Saraf R Ramesh[1]. 1) Department of Studies in Zoology, University of Mysore, Mysore, Karnataka, India; 2) Embryotoxicology, CSIR-Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India.

Comparative reproductive biology has always remained an authentic measure of evolutionary divergence among the species. For such studies, *Drosophila* has always been a favorite model for evolutionary biologists. In the present study, we carried out a comparative analysis of few sexual traits among *D. nasuta* subgroup members to understand their evolutionary divergence. This subgroup is an assemblage of species/subspecies with varying levels of reproductive isolation, and they offer a good model to understand their evolutionary dynamics. We studied mating and re-mating behavior among these members to understand their reproductive biology followed by sperm competition studies. This study revealed their divergence in mating and re-mating behavior. Interestingly, few members of this subgroup exhibited distinctive first male precedence in doubly mated females. In *Drosophila,* male derived seminal molecules influence the reproduction. These molecules are transferred to females during mating along with sperm, wherein they influence the outcome of reproduction. To better understand the role of male derived molecules in reproduction and reproductive isolation among *D. nasuta* subgroup members, we analyzed the dynamics of these molecules after their transfer into the female reproductive tract. Male derived seminal proteins coagulate to form mating plug within the uterus of a mated female. We dissected these mating plug's dynamics among *D. nasuta* subgroup members. Study revealed a significant divergence among the members in their mating plug dynamics, and male mating status significantly influenced the mating plug formation. However, mating plugs formed in inter-specific crosses among the members still reflects their cross-fertility. In spite of the divergence in these molecules, the interaction between the sexes of different subspecies does not come in the way of their cross-fertility. Thus, sexual traits of *D. nasuta* subgroup members offers a measurement of their evolutionary divergence, and reproductive biology of both the sexes contribute towards understanding the evolutionary dynamics of species.

717B

Mapping the genetic basis of wing rowing evolution in *Drosophila yakuba* and *Drosophila santomea* male courtship. Jessica Cande[1], Gordon Berman[2], Ugne Klibaite[2], Benjamin Prud'homme[3], Nicolas Gompel[4], Joshua Shaevitz[2], David Stern[1]. 1) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Institut de Biologie du Développement de Marseille-Luminy (IBDML), Marseille, France; 4) Ludwig Maximilian University, Munich, Germany.

In *Drosophila*, male flies undertake a series of innate, stereotyped courtship behaviors which present female flies with diverse visual, auditory and chemical cues with which to discriminate between potential mates. Innate male courtship evolves rapidly between fly species, and is thought to be an important mechanism for reproductive isolation and species divergence. However, we currently have little understanding, neither at a neurological nor a molecular genetic level, as to the precise mechanisms by which courtship behavior evolves. We are using the *Drosophila yakuba* and *Drosophila santomea*hybridizing species pair as a model system with which to study this problem. These species diverged approximately 500,00 years ago, occupy different habitats, and differ significantly in both morphological characteristics such as adult pigmentation, as well as in behaviors such male courtship song and the frequency of wing rowing during courtship, a behavioral motif in which males rotate their wing blades to face forward and then back of the course of a second. Previously, we were able to use a QTL mapping approach to map wing rowing differences between *D. yakuba* and *D. santomea* to a handful of autosomal loci. Here, we combine an introgression based genetic strategy with automated tracking and motion classification methods in an effort to increase the resolution of our map and identify the genes responsible for evolved wing rowing differences.

718C

A genetic understanding of courtship song variation between Drososophila simulans and Drosophila mauritiana. Yun Ding, Augusto Berrocal, David Stern. Janelia Research Campus, HHMI, Ashburn, VA.

Little is known about the genetic basis of behavior variation among species, especially the types of genes and mutations involved. Song production through wing vibration is a common courtship behavior in Drosophila species and plays important roles in courtship success and species recognition. Though largely stereotyped within species, the song traits vary extensively among species, both qualitatively and quantitatively. We performed quantitative trait locus (QTL) analysis for three courtship song traits between D. simulans and D.mauritiana: Inter-pulse interval, pulse song fundamental frequency, and sine song fundamental frequency (sine FFT), and found distinct QTL landscapes. The sine FFT difference was mapped to a single QTL on the third chromosome. Further fine mapping using over 2000 introgression lines confirmed this QTL and refined it to an interval of 60kb. We are currently making mutants of the candidate genes in the interval for the reciprocal hemizygosity test. .

719A

Genetic determinants of RNA-editing levels of ADAR targets in Drosophila melanogaster. Yerbol Kurmangaliyev, Sammi Ali, Sergey Nuzhdin. Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

ADAR-mediated adenosine deamination (A-to-I editing) is the most common type of RNA-editing in metazoans. Inosines are recognized by translational machinery as guanosines. This may result in changes of primary protein sequences. Several recent studies identified thousands of ADAR editing sites in Drosophila transcriptome. RNA-editing usually affects only fraction of the transcripts, and

the editing level of particular sites may vary strongly. Principles determining specificity of ADAR target sites and their editing levels is poorly understood. Population-level RNA-Seq data allows to study natural variation of various transcriptomic traits. The analysis of associations between this variation and whole genome sequences allows to map putative regulatory variants affecting these traits. Such approaches were successfully utilized to determine quantitative trait loci affecting expression and splicing patterns of the genes. Here we analyzed natural variation of RNA-editing levels in matching genomic and transcriptomic data for 81 natural strains of Drosophila melanogaster. RNA-Seq data was used to detect and quantify genotype-specific editing levels for putative ADAR target sites. Association tests between editing levels and adjacent SNPs revealed multiple RNA-editing quantitative trait loci (reQTL) associated with variations in fractions of the edited transcripts. Analysis of allelic imbalance confirmed that observed changes in RNA-editing levels are regulated by cis-genetic effects. Overall, the detection of reQTL may provide better understanding of mechanisms determining specificity of ADAR substrates. ADAR enzymes are known to target double-stranded regions of RNA. Identified reQTL may represent cis-regulatory elements involved in formations of secondary RNA structures required for ADAR-mediated RNA-editing. To our knowledge, this is the first study of effects of natural genetic variation to the level of A-to-I RNA-editing.

720B

Complex genetic interactions characterize the rapid evolution of genital morphology in *Drosophila*. Stephen R. Frazee, Jessica Pagan, Brittany N. Smith, **John P. Masly**. Department of Biology, University of Oklahoma, Norman, OK.

Understanding the genetic and molecular mechanisms that give rise to phenotypic change is an important goal of evolutionary biology. Male external genitalia represent one of the most rapidly evolving morphological structures observed among internally fertilizing taxa, and thus offer an ideal model to dissect the genetic architecture of divergent structural traits. In *Drosophila*, the male posterior lobes of the genital arch are important mating structures that display dramatic differences in both size and shape among the four closely related species of the *D. melanogaster* species complex. In previous work we identified several small genomic regions that have large effects on posterior lobe morphology when we replaced segments of the *D. sechellia* genome with the homologous regions from *D. mauritiana*. Here, we test the hypothesis that synergistic epistasis among these regions characterizes the rapid evolution of posterior lobe morphology between these species. We phenotyped and genotyped ~3,100 F2 individuals from 11 different pairwise combinations of *D. mauritiana* introgressions that have morphological effects to test the relative importance of genetic additivity versus genetic epistasis in specifying species-specific differences in morphology. We find evidence of both additivity and epistasis, although the direction of the effects is not always consistent with that expected under a history of directional selection. We also find that, on average, the *D. mauritiana* alleles that specify morphology are recessive to those of *D. sechellia*. Our results illustrate a complex genetic architecture underlying this rapidly evolving quantitative phenotype and reveal that the loci specifying posterior lobe morphology appear to participate in a "tug of war" over their effects on morphological variation.

721C

Genetic basis of genital evolution between *Drosophila* species. Maria D. S. Nunes[1], Kentaro Tanaka[1], Corinna Hopfen[3], Matthew Herbert[1], Christian Schloetterer[2], John P. Masly[4], Alistair P. McGregor[1]. 1) Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; 2) Institute of Population Genetics, Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Vienna, Austria; 3) Max Planck Institute for Biology of Ageing, Cologne, Germany; 4) Department of Biology, University of Oklahoma, OK, US.

In *Drosophila*, as in many other organisms with internal fertilization, male genitalia display faster divergence between closely related species than other morphological traits. Phylogenetic and experimental selection studies indicate that a large component of this divergence is driven by sexual selection, however the precise mechanisms leading to differential male reproductive fitness are still a matter of dispute and probably differ among species comparisons. In the *Drosophila simulans* complex, the morphology of several genital and anal structures differs significantly among the three species of the group and they have also been shown to exhibit post-mating prezygotic reproductive isolation. Using QTL and introgression mapping we detected several loci on chromosome 3L and 3R that underlie differences in clasper, posterior lobe and anal plate morphology between *D. mauritiana* and *D. simulans*. Most of these loci affect the trait in the same direction and act additively. However, we also found evidence for epistasis, in particular between the 3rd and the X chromosome. We conducted an RNAi screen in *D. melanogaster* against positional candidate genes located in four regions mapped to high resolution on chromosome 3L and that are differentially expressed either between males and females or between *D. mauritiana* and *D. sechellia*. We found that only seven of these genes have a regulatory role in the development of genitalia. Ongoing genome-editing experiments in *D. mauritiana* and *D. simulans* using the CRISPR-Cas9 system will allow us to identify the evolved genes and possibly the nucleotide changes responsible for the genitalia differences between these two species. Finally, we are also conducting mating experiments to determine if the morphological changes caused by these genes have the potential to elicit reproductive isolation. .

722A

Sexually dimorphic pigmentation in *Drosophila*: lineage sorting and independent comparisons in multiple phenotypic transitions. Sarah Signor[1], Artyom Kopp[2]. 1) Molecular and Computational Biology, University of Southern California, Davis, CA; 2) Evolution and Ecology, University of California, Davis.

Molecular and genetic analysis of phenotypic variation has revealed many examples of evolutionary transitions involving developmental pathways. It has recently become more popular to take a comparative approach to this question, and this work has uncovered some interesting patterns such as the ubiquity of regulatory changes in morphological evolution. Despite the contributions

from these studies it still remains a challenge to understand how many of these traits evolve in general, especially if the question is framed in terms of the exact nucleotide changes involved. Sexually dimorphic pigmentation is a relatively recently evolved phenotype, originating at the base of the *D. melanogaster* group from known modifications to existing enhancers. Since this time there have been multiple gains and/or losses of this phenotype, providing multiple instances for developing a comparative framework as to how the trait evolves. Within the *D. bipectinata* and *D. ercepeae* species subgroup we have investigated the genetic basis of coloration differences in four species pairs that vary with respect to pigmentation phenotype. In three of these crosses we uncovered most or all of the genes responsible for pigmentation differences, including one gene shared between all crosses and a second shared between two. A population genetics analysis of the gene shared between all crosses revealed that in two of the four crosses the genetic basis of the phenotypic difference was the same, and indicated five nucleotide differences responsible for the transition. In the other two crosses the origin of the phenotypic difference is not due to the sorting of an ancestral allele, but appears to have arisen independently. Thus in this species group there have been two transitions due to lineage sorting, involving a relatively small number of nucleotide differences, and two independent transitions in pigmentation phenotype. These four crosses offer a new perspective on the evolution of coloration phenotype, highlighting the importance of lineage sorting *and* the existence of genetic hotspots for specific traits. .

723B

Partial behavioral isolation between two divergent populations of *Drosophila melanogaster* and their mating potential with other wild type populations. Phillip Barnes, Phoebe Winn, Lucy Drayson, Melanie Argueta. Dept Biol, Connecticut Col, New London, CT.

Recent studies have shown significant partial behavioral isolation between two populations, a DDT-resistant strain of *D. melanogaster* (91R) and its unselected control (91C). The partial behavioral isolation is manifested as a significant excess of homotypic and a significant deficiency of heterotypic matings as compared to random mating. Both populations were originally derived from the same foundation population obtained from nature in 1952. Three hypotheses can be proposed for how genetic changes could have occurred to cause the observed partial behavioral isolation between these two strains: 1) behavioral and genetic changes occurred in both strains simultaneously; 2) behavioral and genetic changes occurred primarily in the 91C strain; or 3) behavioral and genetic changes occurred primarily in the 91R strain. Both the 91C and 91R strains were compared to five laboratory wild type "tester" strains by multiple-choice, simultaneous mass-matings. Random mating was consistently observed between 91C and all of the tester strains. However, 91R showed significant partial behavioral isolation (within-group preferences) with some of the tester strains. These results tentatively support Hypothesis 3: that behavioral genetic changes have occurred primarily in 91R. These changes may be associated with the intense selection that occurred for resistance to DDT and/or with possible random genetic changes that could have occurred during bottlenecks of the 91R stock that happened during the artificial selection regime.

724C

Identifying hybrid incompatibility genes in *D. Pseudoobscura* subspecies. Alyssa Black, Nitin Phadnis. Biology, University of Utah, Salt Lake City, UT.

Speciation, the process of one species splitting into two separate species, often involves the evolution of hybrid sterility or hybrid inviability. These reproductive isolating barriers are caused by negative interactions between genes called hybrid incompatibilities. Identifying hybrid incompatibility genes, and understanding their mechanisms, promises to provide fundamental insights into the molecular basis of speciation. A classic example of the earliest stages of speciation are the Bogota and USA subspecies of *D. pseudoobscura*. These young species diverged recently, about 200,000 years ago. Bogota females crossed to USA males produce sterile hybrid F1 males and fertile hybrid F1 females; the reciprocal cross produces all fertile hybrid progeny. Previously, we identified a gene, *Overdrive* (*Ovd*), that is necessary to cause hybrid sterility. *Ovd*, however, does not act alone; additional interacting partners are required. My goal is to identify a large effect *Ovd* interacting partner on the left arm of the X chromosome. To map this gene, we introgressed USA genetic material into a Bogota background near the *yellow* region. All of these *yellow*-introgression lines produce completely fertile hybrid males. Utilizing recombination to shrink this introgression region and using PCR based markers to map the introgression breakpoints, we are narrowing this region to a smaller set of candidate genes. I am generating many more recombinants to gain greater mapping resolution for the hybrid sterility gene. Identifying and characterizing this gene promises to provide insight into the molecular basis of hybrid incompatibilities.

725A

Presence of transposable elements is associated with the expression of hybrid incompatibility in *Drosophila virilis*-*D. lummei* hybrids with and without *Penelope* elements. Dean Castillo, Leonie Moyle. Biology Dept, Indiana University, Bloomington, IN.

Although hybrid incompatibilities are readily observed in interspecies crosses, the genetic basis of most incompatibilities is still unknown. One emerging hypothesis is that hybrid dysfunction is due to a mismatch between parental genomes in selfish elements and the genes that regulate their proliferation; in a hybrid genome, insufficient suppression of selfish elements produces hybrid inviability or sterility phenotypes. Indeed, the few cases of identified hybrid incompatibility genes appear to disproportionately involve loci that regulate selfish/repeat elements. Several recent studies also implicate transposable elements (TEs) as a specific selfish element contributing to hybrid incompatibilities, although in these cases hybrid dysfunction could be explained by additional confounding factors including differences in ploidy. In this study, we more directly evaluated the role of TEs in hybrid incompatibilities by examining hybrids between *Drosophila virilis*, a species that is polymorphic for specific (*Penelope*) TEs, and a closely related species *Drosophila*

lummei that lacks these elements. If TEs influence the expression of hybrid dysfunction, *D. lummei* crosses with *D. virilis* TE-carrying males should show greater hybrid dysfunction than crosses with *D. virilis* males lacking these TEs. Hybrid phenotypes included F1 egg hatchability, total number of F1 progeny, F1 sex ratio, and F1 male gonad dysgenesis. We confirmed that crosses between the *D. virilis* genome strain (TE carrier) and a non-carrying *D. virilis* strain result in the classic 'hybrid dysgenesis' phenotype (atrophied testes when the TE carrier is the father). In contrast, in our interspecies crosses we never observed atrophied testes. We did, however observe significantly reduced egg hatchability in crosses between TE-carrying *D. virilis* and *D. lummei*, compared to a slight effect in crosses where *D. virilis* did not carry TEs. These observations suggest that TEs could contribute to the expression of hybrid incompatibilities via presence/absence polymorphisms, and that the interspecific hybrid phenotype (inviability) differs from the dysgenic phenotype associated with TEs within species (male sterility).

726B

Two loci contribute to a male pheromonal polymorphism associated with ecological adaption in African *D. melanogaster* populations. Henry Chung[1], John E. Pool[2], David W. Loehlin[1], Jocelyn G. Millar[3], Sean B. Carroll[1]. 1) Laboratory of Molecular Biology, University of Wisconsin, Madison, WI; 2) Department of Genetics, University of Wisconsin, Madison, WI; 3) Department of Entomology, University of California, Riverside, CA.

Ecological adaptation that affects traits which are also involved in mate recognition can lead to incipient speciation. However the genetic mechanisms underlying this in diverging populations are not defined. The main cuticular hydrocarbon (CHC) on *Drosophila melanogaster* males is usually 7-tricosene (7-T), a 23 carbon alkene found on the cuticle. Males from West Africa, however, have 7-pentacosene (7-P), a 25 carbon alkene which is associated with thermal tolerance and desiccation resistance. Previous studies associate this polymorphism with incipient speciation in African *D. melanogaster* populations. Using a combination of population genomics and molecular biology, we show that a combination of coding changes in a fatty acid reductase and regulatory changes at a fatty acid elongase contribute to the 7-T/7-P polymorphism in African *D. melanogaster* population. .

727C

Revealing the Secrets of the X: Genomewide Expression Profiles in *Drosophila*. Danielle Herrig[1], Ana Llopart[1,2]. 1) Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA; 2) Biology Department, University of Iowa, Iowa City, IA.

Population genetic models predict that under certain conditions the *X* chromosome will evolve at a faster rate than the autosomes in XY (or ZW) systems (i.e., faster-X effect). Evaluations of protein-coding sequences have indeed shown an excess of divergence on the *X* chromosome compared to autosomes in the *Drosophila yakuba - D. santomea* system, particularly in genes expressed higher in males than in females (i.e., male-biased genes). In addition, whole-genome analyses of gene expression in males of these same species pair indicate that X-linked genes are more differentially expressed between species than autosomal genes. This trend is once again strongest for male-biased genes. However, these studies utilized only males and were therefore limited in their expression profiles. Here, we will investigate whole-genome profiles of *D. yakuba* and *D. santomea* females and males using RNA-seq to further investigate the faster-X effect and its potential consequences on gene expression in hybrids.

728A

Characterizing postzygotic isolation in the *Drosophila nasuta* species complex. Wynn Meyer, Doris Bachtrog. Integrative Biology, University of California, Berkeley, Berkeley, CA.

One route to speciation between populations diverging in allopatry involves the acquisition of hybrid dysgenesis, or postzygotic isolation. Groups of recently diverged species, with limited prezygotic isolation, provide productive models for studying the evolution of these traits, as well as the timescale and genetic changes involved. As a first step towards addressing these questions, we characterize several reproductive isolation phenotypes in members of the *Drosophila nasuta* species subgroup, a complex of 14 species/semispecies with varying but low levels of genetic divergence, many of which are inter-fertile. We first describe variation among parental species in characteristics of courtship song behavior, a set of traits that are known to be species specific in several other groups of Drosophila, but which do not always cause complete prezygotic isolation within the *nasuta* subgroup. We then characterize these behavioral traits in F3 - F20 hybrids derived from large populations of F1 hybrid offspring of several inter-fertile species pairs within the complex. We assess the reproductive success of these hybrids using non-choice assays for success of copulation and measurements of fertility (number of offspring produced). We additionally characterize sex ratios of emerging hybrid offspring across generations, noting several instances in which these ratios have been distorted from those characteristic of the parental species. In combination, these studies suggest that several species within the complex display significant yet incomplete postzygotic isolation, and the variation in phenotypes across individual hybrids suggests that these populations may provide a useful system in which to study the genetic basis of reproductive isolation traits.

729B

Gene Expression Patterns Associated with Sex-Specific Pheromone Production in *Drosophila simulans* and *D. sechellia*. D.R. Swartzlander[1], Jennifer M. Gleason[2]. 1) Molecular Biosciences, Univ of KS, Lawrence, KS; 2) Ecology and Evolutionary Biology, Univ of KS, Lawrence, KS.

Chemosensory signals, including pheromones, influence courtship behavior in *Drosophila*. Differential pheromone production between species can cause failure in mate recognition, thereby leading to reproductive isolation. *Drosophila simulans* males fail to court *D. sechellia* females because of a pheromonal difference in cuticular hydrocarbons (CHCs) between the females. *D. simulans* CHCs

are sexually monomorphic. *D. sechellia* CHCs are dimorphic; males produce similar CHCs to *D. simulans*, but females produce unique CHCs. Quantitative trait loci (QTL) mapping of the species difference identified 3rd chromosome regions that contribute to female-specific CHC production. To identify potentially causative genes, we determined the pattern of gene expression for desaturases and elongases associated with the QTL. Several of these enzymes are associated with sex-specific CHC production in *D. melanogaster*. We predicted that if these same genes influenced the difference between *D. simulans* and *D. sechellia* females, then sex-specific gene expression was expected between *D. sechellia* males and females, but not between the sexes of *D. simulans.* To determine if gene expression correlated with CHC production in the *D. melanogaster* subgroup, our study included an additional monomorphic species, *D. mauritiana* and two additional dimorphic species, *D. melanogaster* and *D. erecta*. Because desaturases and elongases may be involved in other pathways besides CHC production, we extracted RNA from only the abdominal cuticle, the location of CHC synthesis. Genes with differential expression patterns between the sexes were further analyzed by qRT-PCR. Only two genes, *desatF* and *eloF* had female-biased expression in the dimorphic species and were not expressed in the monomorphic species. In *D. simulans-D. sechellia* hybrids, only the *D. sechellia* allele of each gene was expressed, indicating that *cis*-regulatory changes are responsible for the species difference. Further functional studies are needed to confirm the roles of these genes in reproductive isolation.

730C

Phylogenetic Analysis of Abdominal Pigmentation Evolution in the *Drosophila montium* Subgroup. Mubasher Ahmed, Paul Ginsberg, Chenling Antelope, Emily Delaney, Michael Turelli, Artyom Kopp. Department of Evolution and Ecology, University of California, Davis.

Members of the *Drosophila montium* species subgroup exhibit great diversity in patterns of abdominal pigmentation. Females of certain *montium* species exhibit intraspecific pigmentation pattern diversity while males are monomorphic, a phenomenon called sex-limited pigmentation polymorphism. The *montium* subgroup is thus a potential model system for understanding the evolution of sex-specific pigmentation phenotypes. However, the 73 species that make up this group lack a well-resolved phylogeny. Such uncertainty in phylogenetic analysis significantly impacts our understanding of how morphological traits have evolved across this lineage. Here, we construct a phylogeny for 27 species representative of the *montium* subgroup. To this end, we have amplified and sequenced ten nuclear and two mitochondrial loci from each species. We have aligned the sequences and will use the phylogenetics software MrBayes and BUCKy to create a phylogeny using rigorous Bayesian methods. Our phylogeny will allow us to study the evolution of sex-limited pigmentation polymorphism and test for convergent evolution. .

731A

Ectoparasitic mites and their *Drosophila* hosts. Alejandra Pérez-Leaños[1], Mariana Ramirez-Loustalot-Laclette[1], Therese Markow[1,2]. 1) LANGEBIO, CINVESTAV , Irapuato, Guanajuato, México; 2) University of California, San Diego.

Natural populations of the Sonoran Desert endemic *Drosophila nigrospiracula* often are infested with mites from genus *Macrocheles* (Polak and Markow 1995). Originally thought to be strictly phoretic, mites not only affect the reproductive success of flies they are attached to, but it has been demonstrated that they are capable of transmitting infections from one fly to another as well. We have found*Drosophila* individuals from species other than *D. nigrospiracula* infested with mites in different localities in Central Mexico. All *Drosophila* detected with mites are members of the repleta species group. Using molecular methods we were able to identify the mites associated with the flies from central Mexico as members of genus *Dermanyssus,* which belongs to a different family than the Sonoran*Macrocheles* reported by Polak and Markow. Both species of mite are members of order Mesostigmata. Clearly multiple species and families of mites are associated with Mexican *Drosophila* species. We explore the existence of a correlation between species of *Drosophila* and the species of mites that infect them.

732B

Genes caught in the crossfire: Understanding the tension between genome defense and genomic "autoimmunity" by piRNAs. Alexandra Erwin, Michelle Wickersheim, Justin Blumenstiel. University of Kansas, Lawrence, KS.

In sexually reproducing species, natural selection on transposable element (TE) lineages favors high levels of transposition. In response to this threat, piRNA mediated genome defense has evolved and limits the spread of TEs within genomes. However, this means of genome defense comes at a cost - genic off-targeting by piRNAs. Using a dysgenic syndrome in *Drosophila virilis*, we show that patterns of genic off-targeting can differ significantly between strains. Furthermore, in the face of TE activation, off-target effects become increased for certain classes of genes in the dysgenic germline. In light of this form of "genomic autoimmunity", we propose that tension between genome defense and off-target effects is an important determinant in the evolution of the piRNA machinery.

733C

Molecular evolutionary analyses reveals positive selection in the rapidly-evolving synaptonemal complex in the *Drosophila* genus. Lucas Hemmer, Justin Blumenstiel. Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

The synaptonemal complex (SC) is a highly conserved meiotic structure seen across eukaryotes that functions to hold the homologs together during meiosis and facilitate exchange. Five *Drosophila*exclusive proteins have been identified as the components: C(3)G, C(2)M, Cona, Ord, and the newly identified Corolla. Each protein is necessary for proper meiotic function; mutations lead to reduced crossing over and chromosomal non-disjunction. Despite the conserved nature of the SC and the key role that these five proteins have in meiosis in *D. melanogaster*, they display little apparent sequence conservation outside the genus. We have performed a molecular evolutionary analysis to determine the nature of selection that might explain this lack of apparent conservation. Several species

of *Drosophila*have no recognizable sequences corresponding to these crucial SC components and identity declines rapidly in diverged species. SC gene sequences are changing more rapidly than the genome wide average and this can in part be explained by the action of positive selection in almost every SC component, with the exception of C(2)M. Interestingly, across the phylogeny, we find no evidence that changes in the rate of evolution of one component can drive corresponding changes in other components. These analyses are robust to different alignment procedures and sampling across different time scales of divergence. Finally, there is evidence of positive selection at the population level in *D. melanogaster* and *D. simulans* suggesting that adaptation in the SC is ongoing.

734A

Soft shoulders ahead: spurious signatures of soft and partial selective sweeps result from linked hard sweeps. Daniel Schrider[1], Fábio Mendes[2], Matthew Hahn[2], Andrew Kern[1]. 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Biology, Indiana University, Bloomington, IN.

Characterizing the nature of the adaptive process at the genetic level is a central goal for population genetics. In particular, we know little about the sources of adaptive substitution or about the number of adaptive variants currently segregating in nature. Historically, population geneticists have focused attention on the hard sweep model of adaptation in which a *de novo* beneficial mutation arises and rapidly fixes in a population. Recently more attention has been given to soft sweep models, in which alleles that were previously neutral, or nearly so, drift until an environmental shift causes them to become beneficial. It remains an active and difficult problem however to tease apart the telltale signatures of hard vs. soft sweeps in genomic polymorphism data. We show that these two models might not be separable through the use of simple summary statistics. In particular, recombination in regions linked to, but distant from, sites of hard sweeps creates patterns of polymorphism mirroring those expected near soft sweeps. We find that a similar situation arises when using haplotype-based statistics that are aimed at detecting partial or ongoing selective sweeps: it is difficult to distinguish the shoulder of a hard sweep from the center of a partial sweep. While knowing the location of the sweep mitigates this problem, we show that stochasticity in signatures of natural selection will frequently cause the signal to peak far from this site; thus inferences of both the target and the mode of positive selection will often be incorrect. In addition, both the passage of time since a sweep and allelic gene conversion lead to errors in the classification and localization of selective sweeps. This general problem of "soft shoulders" suggests that we currently have only a very limited ability to differentiate soft and partial sweeps from hard sweeps in molecular population genomics data. The soft shoulder effect also implies that the more common hard sweeps have been in recent evolutionary history, the more prevalent spurious signatures of soft or partial sweeps may appear in genome-wide scans.

735B

Ecological adaptations for pigmentation in two colonizing *Drosophila* species: evidence for genotype-environment interaction. Veer Bhan. Department of Biotechnology, UIET, M D University, Rohtak, Haryana, India.

Six altitudinal populations of two sympatric species, *D. melanogaster* and *D. takahashii* were investigated for abdominal pigmentation at rearing temperature (i.e. 20°C) as well as seven different growth temperatures and the shapes of reaction norms were analyzed segment wise for both the species. The darker pigmentation of the abdominal segments decreased according to growth temperatures in agreement with the thermal budget adaptive hypothesis. The shape of the response curves was different between the abdominal segments and for a given segment also different in the two species. Pigmentation score showed clinal increase with altitude in both species. Body size traits also showed higher positive correlations with the abdominal pigmentation in both species. *D. melanogaster* showed higher trait values than *D. takahashii* for both pigmentation as well as body size traits. This suggests an adaptive response resulting in the change of the shape of reactions norm and involved genotype-environment interactions.

736C

The hydrogen isotopes $^{2}H/^{1}H$ involvement across different generations of *Drosophila*: patterns of molecular variation on w^{1118} strain. Gallia Butnaru, Sorina Popescu, Ioan Sarac. Banat Univ Agricultural Sci. "Regele Mihai I al Romaniei" Timisoara, Romania, Box 136, PO1.

The isotopic ratio $^{2}H/^{1}H$ action upon *Drosophila*'s development was evaluated. The amount of ^{2}H (D) used was as in nature (143.4 ppm) and also a lower (30 ppm) and seven higher concentrations, therefore 10 variants being followed on w^{1118} strain. The PS-LAFMCA02-AIHA-01 method was used. In the first cycle the parents [3:3] survived up to 24.45% D concentration. In comparison with females the male individuals pointed out a better viability. In presence of 48.90% D concentration survived 5 and 21 days 16.67% of females and 50.0% of males. Along of 5 generations the high content of D improved the fertility of successive viable descendants**.** Even in presence of D 24.45% the fertility was higher than on control [82.0 ± 9.82 > 41.0±0.71]. The fertility average/5 generations revealed a similar frame [48.4 ±12.4 > 43.7± 9.9]. The content of D in G4 descendants was small (64.2ppm) if in the medium the D concentration was 30 ppm and high (12,404.9 ppm) for the 2.29% D amount. The DNA extracted from adults of five successive generations were used to establish the molecular pattern pattern [4 RAPD and 8 ISSR markers]. The obtained data from the BioDoc Imaging System (UVP) were included in mathematical processing, the similarity coefficient (sc)/correlation (r) were established.. In UPGMA clustering of the 12 molecular markers the similarity vs. variability emphasized 2 groups separated at 0.17 coefficient of similarity. The first and second generations formed the first cluster, the 3rd, 4th and 5th generations being organized in the second one. In the first cluster the variance generated by primers was predominantly without significance [66%].For the DNA extracted from adults of the 5th generation the D content was evaluated. It turned out that the D content in DNA molecule was increased with 1,8 ppm only for the higher concentration of deuterium in medium (24.45% D). We suppose a slowly but significant involvement of D in the genetic background of *D. melanogaster*/w^{1118}strain.

737A

Longitudinal ethanol withdrawal and ethanol intake in outbred *Drosophila melanogaster* raised with and without ethanol-containing food. Kairsten A Fay[1], Alexander Gearhart[1], Megan Garlapow[1], Trudy Mackay[1,2]. 1) Biological Sciences, program in genetics, North Carolina State University, Raleigh, NC; 2) W. M. keck Center for Behavioral Biology, North Carolina State University, Raleigh, 27695.

Alcoholism negatively affects many health parameters in a complex genetic-, sex-, and environmental-dependent manner. Ethanol withdrawal in humans, in particular, results in increased mortality. We explored the effects of acute ethanol withdrawal and longevity in laboratory evolved outbred *Drosophila melanogaster* populations that had been raised on ethanol-containing food (Ethanol-land) or in parallel on regular food (Flyland) for over 90 and over 170 generations, respectively. Both Ethanol-land and Flyland are derived from the same outbred population derived from round-robin mating of 40 inbred lines of the *Drosophila melanogaster* Genetic Reference Panel and maintained by random mating with 800 individuals per generation. We placed Ethanol-land and Flyland flies on ethanol-containing food and regular food. We assayed fifty replicates of three male and three female flies per population per food treatment over six weeks. We also assessed ethanol and sucrose intake in both populations via no-choice and choice Capillary Feeding Assays. These ethanol withdrawal, ethanol exposure, and ethanol intake experiments will enable us to dissect the complex environment, genetic, and sex components affecting mortality, addiction, and intake. The use of *D. melanogaster* to elucidate the complex nature of ethanol intake, chronic ethanol exposure, and ethanol withdrawal allow us to better understand the genetic bases of addiction and withdrawal while eliminating confounding factors found in humans such as obesity or diabetes. .

738B

The genetic basis of phenotypic plasticity in the *Drosophila* energy budget. Elizabeth G. King, Vince S. Farinella, Anna Perinchery, Patrick D. Stanley. Division of Biological Sciences, University of Missouri, Columbia, MO.

The acquisition and subsequent allocation of resources towards different structures and functions affects nearly all organismal traits. Allocation of resources to different traits often shifts significantly in different nutritional conditions. Little is known about the genetic basis of the underlying physiological processes that determine these shifts. We used a multiparental, advanced intercross mapping population, the *Drosophila* Synthetic Population Resource (DSPR), a large set of recombinant inbred lines (RILs) generated from an 8-way, 50 generation cross, to identify the genetic variants determining the four components of the insect energy budget: protein, lipids, sugars, and glycogen in three different nutritional conditions. We reared adult flies resulting from crosses between female RILs and males from a single inbred line on a dietary restriction diet, a control diet, and a high sugar diet. We then used colorimetric assays to determine protein, lipid, glycogen, and sugar content of each sample and subsequently mapped QTL influencing these traits in the different environments. Future work will integrate these energy budget phenotypes with gene expression measurements for key pathways involved in resource allocation. .

739C

Mitochondrial - nuclear interactions mediate genotype-specific transcriptional responses to hypoxia in *Drosophila*. Jim Mossman, Yawei Ge, Nan Li, Zhijin Wu, David Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI.

Mitochondria are involved in almost all cellular processes due to their role in ATP production- providing the majority of the energy currency of cells. However, it is now apparent that mitochondria are important in sensing and mediating signaling pathways that have integrative roles in whole organism phenotypes such as aging, metabolism, disease and other gross measures of fitness. Mitochondria are jointly encoded by both nuclear and mitochondrial genomes, with ~1,200 nuclear genes (nDNA) and 37 mitochondrial genes (mtDNA) required for proper mitochondrial biogenesis; an artifact of their conjoined evolutionary history. Experimental model systems, including *Drosophila*, have shown that fitness-related phenotypes are not the product of either genome in isolation. Instead, it is the epistatic interactions between mtDNA and nDNA (mitonuclear epistasis) that mediates the gamut of phenotypes. It is now apparent that mito-nuclear interactions are modified by environmental input, which has important implications for evolutionary biology and disease genetics. To examine these interactions directly, we tested for the effects of paired combinations of mtDNA and nDNA genomes on gene expression profiles induced by altered hypoxic environments. We disrupted co-evolved mito-nuclear expression in *D. melanogaster* by introducing mtDNA from *D. simulans* onto *D. melanogaster*backgrounds. We used four genotypes: 2 mtDNAs (Dmel and Dsim) placed on two nuclear backgrounds (*OregonR* and *AutW*132). Each genotype was exposed to three hypoxic (6% O_2) environments: (i) 0 minutes (control); (ii) 30 minutes; and (iii) 120 minutes, in a fully-factorial design. Using RNAseq, we found large effects of both nDNA and mtDNA variation on gene expression profiles, and some significant mito-nuclear interactions affecting differential gene expression (e.g. CG11966). Each genotype showed a clear overall response to hypoxia in both sexes. However, we also observed genotype- and sex-specific transcriptional profiles. These results clearly demonstrate there is no single canonical response to hypoxia and that mito-nuclear interactions can strongly influence the transcriptional response to an environmental stressor.

740A

Enhanced sleep is an evolutionarily adaptive response to starvation stress in *Drosophila*. Melissa E Slocumb[1], Josue M Regalado[1], Masato Yoshizawa[3], Greg G Neely[4], Pavel Masek[1], Allen G Gibbs[2], Alex C Keene[1]. 1) Biology, University of Nevada, Reno, Reno, NV; 2) School of Science, University of Nevada, Las Vegas, Las Vegas, NV; 3) Biology, University of Hawai'i, Manoa; 4) Neuroscience Division, Garvan Institution, Sydney Austraulia.

Animals maximize fitness by modulating sleep and foraging strategies in response to changes in nutrient availability. Wild populations

of the fruit fly *Drosophila melanogaster* display highly variable levels of starvation and desiccation resistance. These responses vary with geographic location, nutrient availability, and evolutionary history. Further, flies potently modulate sleep in accordance with food availability, and selection for starvation resistance enhances sleep, revealing strong genetic relationships between sleep and nutrient availability. To determine the genetic and evolutionary relationship between sleep and nutrient deprivation, we assessed sleep in flies selected for desiccation or starvation resistance. While starvation resistant flies have higher levels of triglycerides, desiccation resistant flies have enhanced glycogen stores, suggesting distinct physiological adaptations to food or water scarcity. Strikingly, selection for starvation resistance, but not desiccation resistance, leads to increased sleep, indicating that enhanced sleep is not a generalized consequence of higher energy stores. Thermotolerance is not altered in starvation or desiccation resistant flies, providing further evidence for context-specific adaptation to environmental stressors. F2 hybrid flies were generated from starvation and desiccation resistant lines to examine the functional relationship between nutrient deprivation and sleep. Hybrids exhibit a strong positive correlation between starvation resistance and sleep, while no interaction was detected between desiccation resistance and sleep revealing that prolonged sleep provides an adaptive response to starvation stress. Therefore, we have demonstrated context-specific evolution of enhanced sleep in response to chronic starvation, and we provide a model for understanding the evolutionary relationship between sleep and nutrient availability.

741B

Ontogeny of metabolic rate and mitochondrial physiology in *Drosophila*. Cole Julick, Omera Matoo, **Kristi Montooth**. School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

While scaling laws may well predict metabolic rate as a function of mass across taxa that encompass large differences in body size, metabolic scaling relationships within insect species have recently been shown to vary across development and as a function of the environment (Greenlee, Montooth, Helm. 2014 ICB 54:307). Moreover, there is considerable variation in metabolic rate within species that is independent of variation in mass. For example, we have shown that the scaling of metabolic rate as a function of mass depends on the developmental thermal environment in *Drosophila,* and that mitochondrial-nuclear genotype significantly affects larval metabolic rate. This latter effect is itself conditional on the developmental thermal environment, and we have found that interactions between genotype and developmental environment affect metabolic rate plasticity (i.e., the Q_{10} for metabolic rate) (Hoekstra, Siddiq, Montooth. 2013 Genetics 195: 1129). To better understand the ontogeny of metabolic rate and the underlying mechanisms that govern these patterns, we have characterized larval metabolic rate and aspects of mitochondrial physiology across development for a number of natural *D. melanogaster*genotypes, as well as for mitochondrial-nuclear genotypes that combine naturally occurring polymorphisms from different species to generate energetic inefficiencies. We discuss how changing energy demand in holometabolous insects, as well as tissue-body size allometries, across development may contribute to ontogenetic patterns in whole-organism metabolic rate. In this way development generates a dynamic internal environment that will likely impact the fitness effects of mutations affecting energetic processes.

742C

The investigation of biological effects of Methotrexate and Aminopterin on example of Drosophila melanogaster. O. Antosyuk, A. Marvin, N. Marvin, S. Shihova. Biological sciences, Ural State University, Yekaterinburg, Russian Federation.

Background: It is known that the methotrexate and aminopterin are used actively in medical practice. Methotrexate is inhibitor of dihydrofolate reductase and it disturb a folat`s cycle that involves 1 and 2-chained breaks of DNA, the wrong methylation. And result of this process is carcinogenesis emergence. Aminopterin is enzyme inhibitor by competing for the folate binding site of the enzyme dihydrofolate reductase. In our research were used 3 strains of wild type of *Drosophila melanogaster*: Host(Yekaterinburg, 2005), Belgorod(Belgorod, 2006) and Bios-3(Dvurechensk, 2007). Materials and methods: We determine genome instability and genotoxic effect using methods: 1. Individual fecundity; 2. Frequency of early and late embryonic mortality; 3. Frequency of post embryonic mortality; 4. Frequency of chemomorphoses like «cutting» of the wings; 5. Morphometric analysis of the wings; 6. PCR-analysis of amount copies of *hobo*-element. We used Methotrexate(100;400 μg/kg of medium) and Aminopterin(100; 400 μg/kg of medium) as a factors of chemical stress. Results: The long directed selection was conducted on increasing of apoptosis in the wing`s imaginal discs in the presents of methotrexate every even generation. And «-» selection in the presents of aminopterin led to adaptation formation like in first case. We defined sensitive strains to methotrexate and aminopterin. Now the strain «Bios-3» F_{114} cultivate in the presents of methotrexate for further investigations. Conclusions: Perhaps, different sensitivity of strains formed due to different work of compartments of wing imaginal disc, and also different amount copies of *hobo*-element. Key words: Methotrexate, aminopterin, *hobo*-element, morphometric analysis, dehydrofolate reductase, apoptosis, stress, direct selection.

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743A

Genome-wide test of a life-history model underlying seasonal adaptation in Drosophila. Alan Bergland[1], Subhash Rajpurohit[2], Dmitri Petrov[1], Paul Schmidt[2]. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Biology, University of Pennsylvania, Philadelphia, PA.

Populations of Drosophila living in temperate environments are exposed to variable selection pressures through time and space. Consequently, populations are likely adapt to these heterogeneous selection pressures. Recently, we have shown that hundreds of loci contribute to rapid and cyclic adaptation in response to seasonal fluctuations in selection pressure. We hypothesized that the many of these loci are associated with differential allocation of resources to either somatic maintenance or reproductive output. In this model,

summer favored alleles are associated with increased reproductive output (at the expense of somatic maintenance & stress tolerance) whereas winter favored alleles are associated with increased stress tolerance (at the expense of reproductive output). To assess this hypothesis, we established replicated, out-bred populations of Drosophila and allowed these populations to expand in population size for several generations mimicking population growth during the summer. After population expansion, these populations were exposed to severe starvation/desiccation stress. We have generated allele frequency estimates using informative haplotype priors from samples of flies collected at each generation and at several points in time during the death phase following starvation/desiccation stress. We will contrast allele frequency changes in these experimental populations with observed allele frequency estimates from natural populations sampled during the spring and fall over the course of multiple years and localities. We hypothesize that the summer favored alleles in natural populations will increase in frequency during population growth in our experimental populations and that these same alleles will decrease in frequency during exposure to stress. By assessing this hypothesis genome-wide, we will resolve whether a basic life-history tradeoff between reproductive output and somatic maintenance underlies seasonal adaptation.

744B

Sperm transfer and the enigma of copulation duration in *Drosophila melanogaster*. Cynthia Castro. LANGEBIO, CINVESTAV Irapuato, Irapuato, Guanajuato, Mexico.

Copulation duration, one of the most important elements in mating behavior, shows significant variability among *Drosophila* species. *Drosophila melanogaster* exhibits a twenty-minute copulation duration, however, the timing of sperm transfer during copulation duration in this species is not clear. An early study suggested that sperm transfer did not occur until 10 minutes of copulation had elapsed, while a more recent study argued that it began immediately. To resolve this discrepancy, I examined sperm transfer for two *D. melanogaster* strains from different locations in North America. Experiments showed minimal differences among the two strains, and for both strains, there was no sperm transfer until seven minutes after copulation started.

745C

Wing size evolution in high-altitude Ethiopian *Drosophila melanogaster*: developmental decanalization and the genetics of a complex adaptive trait. Justin Lack, Amir Yassin, John Pool. Genetics, University of Wisconsin-Madison, Madison, WI.

A primary goal in the study of adaptive evolution is to identify genetic changes underlying adaptive phenotypes. While recent advances in genome sequencing have significantly improved this ability, questions still remain concerning how adaptive phenotypes evolve at the genetic level. To improve our ability to detect these loci, we integrated quantitative genetic mapping and population genomics to examine the genetic basis of wing size evolution in high altitude Ethiopian *Drosophila melanogaster*. At over 3000 m in elevation, *D. melanogaster* from the Ethiopian highlands have evolved a total wing area and overall body size significantly larger than any previously examined tropical or temperate population. Cellular level analysis of wing epithelium revealed wing size evolved primarily through increases in cell size. Preliminary results from mapping experiments indicate the genetic basis of wing size evolution is largely distinct from that of body size, with only a single shared QTL peak. To refine the mapping results, we examined all SNPs under each QTL peak for signals of selection and found multiple candidate loci. These candidate loci suggest Insulin/Tor signaling and the Epidermal Growth Factor Receptor (EGFR) pathway were both responsible for overall body size shifts, but subsequent changes in the EGFR pathway occurred to further increase wing size and augment wing shape. In addition to size variation, large-winged Ethiopian lines possess a large number of wing vein malformations (>30% of lines exhibit dramatic, heritable vein developmental abnormalities) relative to other African populations (<1% of Zambian lines exhibit these phenotypes). This suggests that a consequence of dramatic wing-size changes has been the decanalization of wing developmental pathways. We conducted large-scale mutagenesis of these Ethiopian and Zambian lines to test this hypothesis. Mutagenized Ethiopian lines that previously had never produced wing vein mutants, produced significantly more vein mutants than similarly treated Zambian lines, suggesting wing development in large-winged Ethiopian flies is much more perturbable relative to that of more typical Sub-Saharan African *D. melanogaster*.

746A

Mutational paths to repeated evolution of Alcohol Dehydrogenase gene function. David Loehlin, Sean Carroll. Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

How is a gene's function and structure shaped by evolution? *Alcohol dehydrogenase* (*Adh*) activity has evolved repeatedly among *Drosophila* species, offering a tractable model of gene function evolution. Dissecting these repeated shifts in gene function might reveal common elements which natural selection has used to shape gene structure. To accomplish this, I am mapping the function-altering mutations to the *Adh* gene across several lineages using a transgenic genetic mapping approach. I find that *Adh* alleles inserted into the same AttP site correctly recapitulate endogenous enzyme activity differences. These biochemical differences appear to have fitness consequences: transgenic flies carrying different *D. melanogaster Adh* alleles (the classic fast/slow polymorphism) have substantial differences in alcohol resistance. In the *D. virilis-americana* lineage, tandem gene duplication - apart from sequence divergence – appears to have an unexpectedly large, non-additive effect on enzyme activity. This indicates that duplication events, even before additional substitutions, could be a mutational path to large phenotypic changes.

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747B

Functional evolution of alcohol dehydrogeanse in *Drosophila*. Mohammad Siddiq, Joe Thornton. Ecology and Evolution, University of Chicago, Chicago, IL.

A goal in evolutionary genetics is to understand how changes at the sequence level produce changes in function and fitness. The *Drosophila* ADH protein provides a classic and tractable model for studying this issue: population genetic analysis of the *Adh* coding sequence indicated adaptive evolution, a hypothesis that is concordant with the expansion of *D. melanogaster* into alcohol-rich environments. However, functional assays are necessary to establish whether the molecular signature of selection on *Adh* is causally related or merely correlated to the increased fitness of *D. melanogaster* in fermenting environments. We have therefore traced the evolution of ADH function in flies of the *D. melanogaster subgroup* using ancestral reconstruction and biochemical assays. In spite of ADH's essentiality in ethanol catabolism, we find that the protein's ethanol activity has not changed in *D. melanogaster*. The discordance between the long-held hypothesis of adaptive evolution and our functional data broadly raises questions about our ability to ascertain adaptation from correlative evidence in absence of empirical characterization.

748C

The genomics of parthenogenesis: Insight from the facultative parthenogenetic fly *Drosophila mercatorum*. Craig Stanley[1,6], Danny Miller[2,3], Therese Markow[4,5], R. Scott Hawley[2,3], Rob Kulathinal[1,6]. 1) Department of Biology, Temple University, Philadelphia, PA, USA; 2) Stowers Institute for Medical Research, Kansas City, MO, USA; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA; 4) Laboratorio Nacional de Genomica de la Biodiversidad, CINVESTAV, Irapuato, Guanajuato CP, Mexico; 5) Univserity of California San Diego, San Diego California, La Jolla, CA, USA; 6) Center for Computational Genetics and Genomics, Temple University, Philadelphia, PA, USA.

Parthenogenesis, or reproduction without fertilization, provides a "last resort" method of reproduction when the availability of males is low, resulting in impaternate offspring. Parthenogenesis is seen in several classes of organisms, such as reptiles and fish, but is known to occur consistently in only two *Drosophila* species, *Drosophila mercatorum* and *Drosophila mangabeirai* . Although no stocks of *D. mangabeirai* exist outside of wild populations, there are several lab strains of both parthenogenetic and sexually reproducing *D. mercatorum*. While several hypotheses have been proposed regarding the allelic diversity necessary for parthenogenesis to occur in *D. mercatorum*, the underlying genomic basis for the phenomenon remains unknown. Using a combination of Illumina short-read and Pacific Biosciences long-read sequencing, we have generated a draft genome of *D. mercatorum*. Here, we provide initial characterizations of the genomes of both sexual and parthenogenetic strains of *D. mercatorum*. We identify genomic variants differentiating sexual and parthenogenetic strains and discuss the potential contribution of these variants to the parthenogenetic process.

749A

The genetic basis of a female-limited pigmentation polymorphism in the *Drosophila montium* subgroup. Emily K. Delaney[1], Thaddeus Seher[1], Nicholas Appleton[2], Steve Chenoweth[2], Artyom Kopp[1]. 1) Evolution and Ecology, University of California-Davis, Davis, CA; 2) University of Queensland, Australia.

Despite the fact that males and females share a genome, sexes can differ in expression of sexually dimorphic traits. In some cases, these traits can also be polymorphic, where multiple genotypes are expressed as two or more morphs in one sex but not the other. Identifying the genes underlying these sex-limited polymorphisms, especially when they are controlled by autosomal loci, can help clarify how sex-specific gene regulation evolves for dimorphic traits. We are studying one such sex-limited trait—female-limited abdominal pigmentation polymorphism—in *Drosophila serrata*, a member of the*montium* subgroup. In this species, females are polymorphic for light and dark pigmentation whereas males always show monomorphic light pigmentation. We used crosses to establish that abdominal pigmentation in this species is controlled by a single Mendelian locus with a dominant dark allele. We then introgressed the dark phenotype into a light strain and identified a ~700 kb candidate region on the Muller C-element (chromosome arm 2R) associated with female pigmentation. Surprisingly, this region contains no known pigmentation genes. To identify causal variants within this locus, we have sequenced light and dark pools of wild caught flies and tested for associations with abdominal pigmentation. Our results will identify a novel component of the *Drosophila* pigmentation pathway and the genomic regions involved in producing a sex-limited trait.

750B

Quantitative genetics of food intake in *Drosophila melanogaster*. Megan Garlapow, Trudy Mackay. Department of Biological Sciences, Program in Genetics and W. M Keck Center for Behavioral Biology, NC State University, Raleigh, NC 27695-7614.

Food intake is an essential animal activity, regulated by signaling pathways, nutrient perception, taste perception, and other processes. To identify factors associated with food intake and its variation in a model system, we performed genome wide association studies (GWAS) in the *Drosophila melanogaster* Genetic Reference Panel (DGRP). The DGRP population consists of 205 wild-derived, inbred, fully sequenced *D. melanogaster* lines, enabling GWAS where all variants are known. We assessed food intake in 182 DGRP lines, finding highly significant genetic variation among the lines, sexual dimorphism in feeding behavior, and genetic variation in sexual dimorphism. The top hits were significantly enriched for components of the *Epidermal Growth Factor* signaling (*EGF*s) pathway. The role of*EGF*s in invertebrate food intake remains largely unexplored. Next we computed the coefficient of environmental variation (CV_E) of food intake within each DGRP line. CV_E quantifies the variability of food intake between individuals in genetically uniform lines. We performed a GWAS of CV_E and found significant genetic variation and a distinct genetic basis from average food intake, indicating genetic control of variance in food intake. Individuals within some DGRP lines consistently consume similar amounts of food, while other lines' individuals consume widely varying volumes of food. Validation experiments using RNAi mutants have enabled us to confirm genes affecting food intake and CV_E of food intake that previously had not been described in *D. melanogaster* while DGRP-based SNP confirmations have

allowed us to confirm individual SNPs predicted by the GWAS. The DGRP allows us to explore the fundamental genetic underpinnings of food intake and its CV_E in a genetically tractable population.

751C

Using artificial selection to validate single nucleotide polymorphisms affecting sleep duration in *D. melanogaster*. Susan Harbison[1], Yazmin Serrano Negron[1], Nancy Hansen[2], Amanda Lobell[1]. 1) Laboratory of Systems Genetics, NHLBI, Bethesda, MD; 2) The NISC Consortium, NHGRI, Bethesda, MD.

Despite mounting evidence that sleep disorders and voluntary sleep loss contribute to an increased risk of human metabolic and cardiovascular disease, the physiological purpose of sleep remains elusive. Mammalian sleep characteristics are conserved in *Drosophila*, allowing us to employ this genetically tractable model organism in the study of sleep. In a previous experiment, we identified 1,190 candidate Single Nucleotide Polymorphisms (SNPs) associated with night sleep duration using the fully sequenced *Drosophila* Genetic Reference Panel (DGRP). Validating the role of these SNPs in sleep is challenging; however, it may be possible to confirm these candidate polymorphisms via artificial selection of a mixed population that we constructed from 10 long- and short-sleeping lines of the DGRP. We subjected replicate populations to selection for long and short night sleep. The response to selection was rapid; after 13 generations, night sleep diverged by 10 hours on average. Day sleep duration had a significant correlated response to selection, and the response was in the same direction as night sleep. We examined changes in the underlying allele frequencies across the genome during selection by sequencing PCR-free DNA libraries of groups of flies from each of the four selection populations over seven generations. We also sequenced DNA from two additional contemporaneous unselected populations. Preliminary results indicate that the allele frequencies of 204 previously identified SNPs changed linearly over time with the artificial selection procedure, and 49 SNPs exhibited a linear trend in one sex only. Allele frequencies changed in the unselected populations as well, but the changes were in different SNPs, of low magnitude, and largely confined to a region in linkage disequilibrium on chromosome *3L*. These results suggest that a portion of the SNPs identified in the GWAS contribute directly to changes in sleep duration. Additional analyses will address the role of all SNPs in the genome. .

752A

A Hidden Markov Model for testing GWAS association by the aggregate signal of multiple linked SNPs. Ziyad Knio, Keegan Kelsey, Andrew Clark. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Genome-wide association studies typically use single marker association (SMA) tests to statistically identify variants that may contribute to observed variance of a trait. SMA tests are relatively low in statistical power in Drosophila studies where typically fewer than 200 lines are tested. Here, we use a Hidden Markov Model (HMM) and incorporate information from multiple contiguous variants and the population LD to identify whether a given region shows an enrichment of unlinked, marginally significant SMA values, identifying regions that would otherwise escape detection under conservative genome-wide thresholds of significance. We scored position effect variegation (PEV) in 103 lines of the Drosophila Genetic Reference Panel and fitted a HMM to resulting SMA values. The HMM uses *p*-values from a genome-wide set of SMA values, and modifies the probability of transitioning from state to state between adjacent variants using linkage disequilibrium (LD) values, measured as r^2. The model then assigns each variant to one of two hidden states, "interest" or "non-interest," resulting in an output of regions that vary in genomic length, number of variants, and posterior probability. Regions of "interest" are further assigned a combined regional *p*-value and compared to a null distribution generated by randomly shuffling phenotypes relative to genotypes. The HMM successfully returned a region that is a well-known modifier of the PEV phenotype, *JIL-1* (*p*-value < 0.001), among other known and novel QTLs. Applying HMMs to GWA studies is particularly useful because it can be used to recover broad regions in which no single variant attains statistical significance, but the aggregate association of many nearby independent variants may be highly improbable. This is of interest in Drosophila studies, where average LD is less than average gene length and most published GWA studies are limited in power to identify causal loci. Future applications include fitting the HMM to results of other published GWAS and assessing added value in non-Drosophila species. In addition, we will test HMM-identified regions with functional follow-up using RNAi and deletion stocks. Finally, we plan to release a computational tool for general use.

753B

Investigating the female's role in sperm competition in *Drosophila melanogaster*. Simone White, Jessica Sitnik, Clement Chow, Andrew Clark, Mariana Wolfner. Molecular Biology & Genetics, Cornell University, Ithaca, NY.

The formation of a fertilized egg involves many interactions between males, females, and their gametes. This is even more complex in cases of multiple mating, as in *Drosophila melanogaster,* as the presence of ejaculates from multiple males presents the opportunity for sperm competition. Male seminal fluid proteins are known to influence sperm competition outcomes, in addition to other post-mating effects. While studies have shown that female genotype is also important for sperm competition outcome, the mechanisms underlying the female's contribution to the success of a particular male's sperm are less understood. A previous GWAS screen based on sperm competition phenotypes across natural isolates of *D. melanogaster* identified candidate genes for the female's contribution to sperm competition. Interestingly, many of these genes have predicted neurological function, pointing to an active role for the female in sperm use and preference. We performed ubiquitous or tissue-targeted local RNAi knockdowns to assess the impact of decreased expression of these genes on sperm competition. Females were scored for sperm competition effects using progeny-phenotype assays, and for reproductive processes including fecundity, fertility, and resistance to remating. Of 11 genes tested so far, knockdown of 5 affected sperm competition outcomes; tests of 5 additional genes are in progress. Genes whose knockdowns in females affect sperm competition outcomes will be further characterized for their effects on sperm storage, retention and release, and on sperm competition,

by direct examination of sperm dynamics within the female, and for the neurons (or other tissues) through which these genes exert their effects. We thank the NIH for funding (R01-HD059060).

754C

***Drosophila* visual receptor diversity through the lens of the i5K project. Markus Friedrich**[1], Jeffery Jones[2], Megan Porter[3], Daniel Hughes[4], Shwetha Murali[4], Kim Worley[4], Richard Gibbs[4], Stephen Richards[4]. 1) Dept Biological Sciences, Wayne State University, Detroit, MI; 2) UC Department of Pediatrics, Cincinnati Children's Hospital Medical Center, OH; 3) Department of Biology, University of South Dakota, Vermillion, SD; 4) Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The visual behavior of the fruitfly is mediated by a diversity of light receptors, which includes seven paralogs of the opsin gene family (LWS: Rh1, Rh2, Rh6; SWS-B: Rh5; SWS-UV: Rh3, Rh4; uncharacterized Rh7). Many of the *Drosophila* visual receptor genes are well known to be ancient, but a comprehensive picture of the phylogenetic depth represented by these genes with regards to the visual evolution of arthropods is missing. We report the results from exploring the conservation of opsin genes in genomic data that have become available through the 5000 arthropod genomes initiative (i5K). Analysis of 93 novel i5K opsins together with 889 previously published metazoan opsins and 93 additional recently published opsins leads to the following main insights: (1) The LWS, SWS-B, and SWS-UV opsin subfamilies are ancient, predating the origin of insects and Crustaceans; (2) The still enigmatic Rh7 opsin is also ancient, being conserved throughout insects, Crustaceans and Chelicerates; (3) The *Drosophila* genome is missing two ancient extraretinal opsin subfamilies: arthropsin and c-opsin. The recently described arthropsin subfamily is present in winged insects including dragonflies and true bugs but was lost during early evolution of indirect developing insects (Holometabola), while the c-opsin family has been lost even more recently during the early evolution of flies in the lineage leading to*Drosophila*. In summary, *Drosophila* harbors the complete diversity of insect retinal opsins, and one putative ancient extraretinal opsin (Rh7), which still awaits functional characterization. The functional study of the deeply conserved, extraretinal c-opsin and arthropsin subfamilies will depend on studies in satellite insect model organisms given their lack in *Drosophila*.

755A

Investigating sexual conflict in the facultative parthenogenetic species, *Drosophila mercatorum*. Nichole Rigby, Rob Kulathinal. Department of Biology, Temple University, Philadelphia, PA.

Sexual conflict—the presence of opposing fitness strategies in males and females—provides an important evolutionary mechanism that can drive rapid divergence between sexes and species. This conflict typically results in an adaptation in one sex that adversely affects the fitness of the opposite sex. As a result, males and females coevolve in a dynamic, evolutionary "arms race." The availability of female-only lines from the facultative parthenogenetic species, *Drosophila mercatorum*, provides a unique opportunity to study sexual conflict without the application of artificial selection over successive generations. Here, using sexual and parthenogenetic lines of *D. mercatorum* we implement high-throughput behavioral and mate choice assays to study the long-term evolutionary effects of sexual conflict. Specifically, we test whether differences in behavior between sexual and parthenogenetic lines closely mirror those found in previous studies using artificially-selected lines in *D. melanogaster*, but on a longer evolutionary time-scale.

756B

The importance of forest patches to the maintenance of genetic diversity of *Drosophila* in the Brazilian pampas. Vera Valente, Jean Lucas Poppe, Hermes José Schmitz. GENETICA, UFRGS, Porto Alegre, Porto Alegre RS, Brazil.

The species composition and the relative abundance of species in an insect community can vary in time and space for many reasons, including climatic variables and habitat preferences. Drosophilids were collected each quarter from April 2011 to April 2012 (five collections in all) in a natural area of the Pampa biome (Brazil), considering three environments: open field, forest edge and the interior of forest patches. A Kruskal-Wallis test and a chi-square test were used to examine the effects of temporal and spatial components on the drosophilid assemblage. The abundance of both Neotropical and exotic species was affected by temporal and spatial components. The species of the *D. repleta* group were predominantly more abundant in the open field, but the species of this group migrated to the forest patches during periods of thermal stress. The distribution pattern and the invasive behavior of drosophilids are related to the thermal tolerance of each species under genetic influence. Forest patches appear to act as a center of recolonization, reinforcing their importance in the maintenance of biodiversity in the Pampas, including genetic diversity, and favoring the occurrence of some species in open environments. Keywords: Biodiversity; behavior; Drosophilidae; Neotropical; Pampas; seasonality.

757C

Detecting meiotic drive by whole-genome sequencing of pooled embryos. Kevin Wei, Shuqing Ji, Jimin Lee, Deanna Lin, Chandramouli Rathnam, Andrew Clark, Daniel Barbash. Molecular Biology and Genetics, Cornell, Ithaca, NY.

Oogensis is asymmetric, producing only one oocyte from four meiotic products. Any chromosomal element that biases meiotic segregation can therefore effectively increase its transmission rate throughout the population. Centromeres and telomeres have been proposed as hotspots of meiotic drive, but very few cases of meiotic drive have been identified. Typically, drive is detected by generating F1 hybrids with and without the suspected driver. The segregation frequency of the heterozygous chromosomes in the F1 females can then be assayed by genotyping the F2 progeny. However, due to sampling error, the power of detection, particularly for weak drivers, requires genotyping very large numbers of progeny. Here, we present a method to determine segregation frequencies by whole-genome sequencing of pools of thousands of F2 embryos. Embryos were collected at 2-3 hrs to minimize potential viability

differences between the genotypes. Allele frequencies were estimated at heterozygous SNP sites genome-wide and tested for deviation from Mendelian frequencies. Due to recombination, the signal of distortion is expected to attenuate around the causal loci, dropping to zero at 50 cM. Using this approach, we tested whether long telomeres could bias segregation frequencies by mating either mutant strains that have accumulated long telomeres (*Gaiano-III* and *Hmr*$^{-}$) or a natural line with long telomeres from the DGRP to DGRP lines with short telomeres. We found no evidence for a strong segregation deviation of long telomeres. To make a confident call on weaker drive effects we are developing statistical models that account for the overdispersion of the data, developmental differences among progeny, and the Mendelian distortions caused by viability effects, and can achieve greater power by accommodating the signal of many linked SNPs.

758A

Localised control of Torso receptor tyrosine kinase activation in *Drosophila* terminal patterning. Travis Johnson[1,2], Michelle Henstridge[1,2], James Whisstock[1,3], Coral Warr[2]. 1) Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; 2) School of Biological Sciences, Monash University, Clayton, Victoria, Australia; 3) Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Victoria, Australia.

In *Drosophila,* specification of the early embryo termini is governed by localised activation of the Torso receptor tyrosine kinase (RTK). This is achieved by an unknown mechanism that involves the perforin-like protein Torso-like; the only known terminal patterning factor restricted to the embryo poles. Torso-like has long been postulated to regulate Torso activity via localised proteolytic cleavage of the growth factor Trunk. By visualising Trunk localisation in live embryos we find that it is not ubiquitously present in the extracellular space surrounding the embryo, but rather is found only at the embryo poles. Strikingly, this localisation is highly dependent on Torso-like, suggesting that local Trunk secretion may be the mechanism that restricts Torso activation to the termini. Together, these data represent a new paradigm for the spatial control of receptor tyrosine kinase signalling by localised growth factor secretion, and define a new role for perforin-like proteins in eukaryotes.

759B

Maternal *lolal* regulates Dpp and Screw transcription in dorsal/ventral patterning. Janine C Quijano[1], Osamu Shimmi[2], Theodor Haerry[3], Stuart Newfeld[1]. 1) Arizona State University, Tempe, AZ; 2) Institute of Biotechnology, University of Helsinki, Helsinki Finland; 3) Center for Molecular Biology & Biotechnology, Florida Atlantic University, Boca Raton, FL.

Genetic screens for dominant maternal enhancers of *dpp* recessive lethal mutations have been successful in identifying signal transduction pathway components essential for dorsal/ventral axis formation in the embryo. However, no factor controlling transcriptional activation of *dpp* or its fellow BMP family member *screw*, during this process, has been unidentified. Here we report that a dominant maternal enhancement screen identified *lolal,* a gene encoding a BTB-domain chromatin binding protein. Embryos derived from enhancement crosses display a ventralized phenotype that is fully rescued by maternal expression of constitutively active Dpp receptors (Thickveins and Saxophone) or the signal transducer Medea. Examination of germline clone embryos that are maternal and zygotic *lolal* mutants revealed a reduction in the level of *dpp* yet an increase in *screw* transcription. Unbalanced expression of these two morphogens did not visibly affect pMad expression, but these embryos contain a significantly reduced region of *zen* expression (*zen* is a direct target of Dpp/Screw signaling) consistent with the ventralization of *dpp* maternally enhanced embryos.
Alternatively, *twist* expression was unaffected in these embryos suggesting that *lolal* is not a general regulator of early zygotic transcription. Collectively, the data reinforce the value of screens in Drosophila as a premier method for gene discovery and identify *lolal* as the first gene shown to be necessary for BMP transcription during dorsal/ventral patterning in any organism.

760C

A gene expression approach to identify novel posterior signaling genes. Julia Wittes, Trudi Schüpbach. Department of Molecular Biology, Princeton University, Princeton, NJ.

During *Drosophila* oogenesis, signaling between the germline and soma pattern the egg chamber and establish the future embryonic axes. It is known that at mid-oogenesis, a subset of somatic epithelial cells called the posterior follicle cells (PFCs) generate a 'posterior signal' or 'polarizing signal' that produces cytoskeletal reorganization in the oocyte, causes the anteriorward migration of the oocyte nucleus, and ultimately generates the proper localization of the polarity determinants *bicoid* and *oskar*. Traditional forward genetic approaches have identified several genes required for posterior signaling, but the identity of the signal itself remains unknown. To find genes involved in posterior signaling, we designed a screen to identify genes more highly expressed in the PFCs than in the rest of the ovary. We genetically created egg chambers with ectopic PFCs or without PFCs using the UAS-GAL4 system. Microarray analysis was then used to identify ~75 genes whose expression increases when ectopic PFCs are induced and decreases when PFC specification is disrupted. These genes were screened for involvement in posterior signaling using RNAi. A number of promising posterior signaling candidates have been identified using this gene expression approach.

761A

Dpp Signaling Directs Nuclear Migration to its Source in the Blastoderm Embryo. Yongqiang Xue, Juan Chahda, Claudia Mizutani. Biology Department, Case western Reserve University, Cleveland, OH.

During the *Drosophila* blastoderm development, thousands of nuclei acquire distinct transcriptional states in response to morphogen gradients. Even though this stage is generally considered to be static due to the lack of major movements prior to gastrulation, previous work had found that the nuclei migrate throughout the blastoderm stage and generate a stereotyped density profile along the

embryonic axes. Alterations in the maternal gradients Bicoid and Dorsal were shown to disrupt the normal nuclear density pattern and affect the position of pair-rule gene expression stripes along the axes. Here, we investigate if the effect observed for Dorsal occurs indirectly via Dpp/BMP signaling. We reasoned that this could be the case since the formation of the Dpp gradient is regulated by Dorsal and because there is a significant nuclear movement towards the dorsal midline where peak levels of Dpp are present. We show that embryos that are mutant for *dpp*, the asymmetric nuclear density along the dorso-ventral axis is abolished despite the presence of a normal Dorsal gradient. We also created genotypes that modify the Dpp gradient to test the extent to which Dpp signaling can orchestrate nuclear migration. Finally, we made a survey of candidate genes known to interact with the cytoskeleton that are expressed either dorsally or ventrally during early embryonic stages and found *frazzled*, *dreadlocks* and *GUK-holder*. We are currently testing if these genes respond to the Dpp signaling pathway and if their mutations cause defects in nuclear migration. Our results may provide novel insights on how morphogens regulate cellular movements and form-generating processes in addition to their well-studied dose-dependent regulation of gene expression. The involvement of Dpp signaling in nuclear migration may also be relevant to many other developmental contexts that rely on its gradient.

762B

A dual-function motif explains dynamic positional specification in *Drosophila melanogaster*. Berta Verd[1], Anton Crombach[1], Nick Monk[2], Johannes Jaeger[1]. 1) Centre for Genomic Regulation (CRG). C/ Dr. Aiguader 88, PRBB Building, 08003 Barcelona, Spain; 2) School of Mathematics and Statistics and Centre for Membrane Interactions and Dynamics. University of Sheffield. Hicks Building, Sheffield, S3 7RH, UK.

Insects use two main modes of segmentation during development: the more ancestral short-germband mode (eg. *Gryllus bimaculatus*), where new segments form sequentially, and the long-germband mode (eg. *Drosophila melanogaster*) where all segments form simultaneously. In dipterans, where the long-germband mode of segmentation is used, the gap genes are activated by maternal gradients and cross regulate each other to form the first zygotic regulatory layer of the segmentation gene hierarchy. A precise mathematical model of the gap genes in *Drosophila melanogaster* was obtained from quantitative spatio-temporal expression data and used to study the dynamics of pattern formation. This approach showed that two distinct dynamical regimes govern anterior and posterior trunk patterning and, most surprisingly, that posterior gene expression dynamics result from an underlying damped oscillator molecular mechanism. The mysterious anterior shifts of posterior gap gene domains can now be explained as an emergent property of this. A dual-function three-gene motif embedded in the gap gene regulatory network was identified as sufficient to recover both anterior and posterior dynamical regimes; which one governs a given region depends on the genes involved. This motif is known as the AC/DC circuit. The dynamical repertoire of this motif consists of only one more possible regime, this one not found in the gap gene system: oscillations. Since molecular oscillations are characteristic of short-germband segmentation, these findings further connect both segmentation modes suggesting that they might have more in common than previously thought, helping us conceptualise and better understand the evolution of the various modes of segmentation in insects. .

763C

Pax6 and the Polycomb Repressive Complex Promote Eye Formation by Repressing Alternate Non-Ocular Fates. Jinjin Zhu, Justin Kumar. Department of Biology, Indiana University Bloomington, Bloomington, IN.

Polycomb Group (PcG) proteins play important roles in transcriptional repression during normal development. In *Drosophila*, reductions in PcG protein levels can, among other things, induce inappropriate Hox gene expression and force homeotic transformations of the wing, leg and antenna. Despite the importance of the PcG proteins in regulating Hox gene expression, its relationship to endogenous gene regulatory networks in tissue/organ fate specification is not well understood. We have recently discovered that the *Drosophila* Pho-repressive complex (PhoRC) cooperates with Pax6 proteins to suppress the establishment of non-ocular fates in the developing eye. Pho-repressive complex (PhoRC) is a PcG complex that contains the only sequence-specific DNA binding protein *Pleihomeotic* (*Pho*), which recognizes Polycomb Response Elements (PREs), and *Scm-related gene containing four mbt domains* (*Sfmbt*). PhoRC is involved in the recruitment of Polycomb Repressive Complex 1 (PRC1) and 2 (PRC2) to PREs. Once recruited to target sites, PcG proteins add and recognize inhibitory marks on histone proteins to repress target gene expression. The Pax6 genes *twin of eyeless* (*toy*) and *eyeless*(*ey*) are members of the evolutionarily conserved retinal determination gene regulatory network. Head defects, a block in eye specification, and a homeotic transformation of the eye into head epidermis characterize loss-of-function Pax6 mutants. Conversely, overexpression of either gene induces ectopic eye formation in non-ocular tissues. These features place both Pax6 genes atop a hierarchy that controls eye specification. We discovered that the simultaneous reduction of Pax6 and PhoRC promotes the de-repression of the Hox gene *Antennapedia* (*Antp*) and the wing selector gene *vestigial* (*vg*). As a result, the eye undergoes a homeotic transformation into a wing. These mutant discs also undergo hyperplastic growth and can achieve a size that is several times greater than normal. Our results suggest that the Pax6 and the Polycomb Repressor Complex cooperate to control growth of the eye disc and prevents the establishment of wing fates. We will discuss the mechanisms by which alternate fates are prevented from taking hold in the developing eye.

764A

Eyeless Participates in the Establishment and Maintenance of Dorsal-Ventral Patterning Within the Developing Eye. Luke R. Baker, Bonnie M. Weasner, Justin P. Kumar. Dept Biol, Indiana University, Bloomington, IN.

Pax6 transcription factors control eye development in all seeing animals. The *Drosophila* genome contains two Pax6 genes: *twin of eyeless* (*toy*) and *eyeless* (*ey*). Both genes are members of an evolutionarily conserved retinal determination network. Loss of *ey* results in

a severe reduction in the size of the compound eye while ectopic expression of either gene is sufficient to induce eye development in non-ocular tissue. The *ey[2]* mutant harbors a transposable element that has been inserted within an eye specific enhancer. The size of the adult eye in these mutants is variable and range from having a full complement of ~800 ommatidia to lacking any obvious retinal tissue. Though previous studies have suggested that *ey* functions solely to specify eye development, it is currently thought that the small to moderate eyes are the result of Toy compensating for the lack of Ey protein. Furthermore, it has been assumed that the retinal tissue that does develop in these mutants was likely to be normal in all respects except for the overall reduction in size of the adult eye. We have recently examined the small to moderate eyes that characterizes the majority of *ey[2]* adult flies and report an exciting new role for *ey* in retinal development. Our analysis of developing and adult retinas indicates that ventrally fated tissue is preferentially lost in *ey* mutants. At one extreme are the small-eyed *ey[2]* adult eyes which are made exclusively of dorsally fated tissue while *ey[2]* flies with medium to large eyes have increasing amounts of ventrally fated tissue. These results suggest that Ey participates in either establishing or maintaining dorsal-ventral compartments in the eye. We have also examined the developing retinas of *sine oculis* (*so*) and *eyes absent* (*eya*) mutants and observe that in these mutants the retinal field appears to be composed almost entirely of dorsally fated tissue. These two genes are also members of the retinal determination network and their expression is dependent upon Ey. These results suggest that the retinal determination network may function as a whole to promote dorsal-ventral patterning of the eye field. If correct, this represents a new and exciting function for this gene regulatory network. .

765B

Putting the Teashirt Family of Genes into context: Their role in Eye Development and Tissue Fate Segregation. Sneha Palliyil and Justin P. Kumar, Department of Biology, Indiana University. Sneha Palliyil, Justin Kumar. Bio Dept, IU, Bloomington.

Primordial tissues of two different organs can be juxtaposed during the primary stages of development. In these instances, their fates will be controlled by very distinct gene regulatory networks(GRNs).A growing body of evidence indicates that adjacently expressed GRNs often have mutually antagonizing relationships such that each network antagonizes the expression of the other. Eye development in both flies and vertebrates is an excellent model to understand the relationship between such GRNs. Work from several labs has shown that the Sine Oculis(So) and Eyes Absent(Eya) proteins, which are members of the retinal determination (RD)network, function to repress the expression of GRNs that control surrounding non-ocular fates like the antenna, maxillary palp and head epidermis. Similarly in vertebrates the loss of mouse LIM protein,Lhx2 and frog Rax leads to the de-repression of brain specific genes in the eye field. We are interested in understanding the full relationship between the RD and surrounding networks. We have evidence to suggest that two additional RD proteins, Teashirt(Tsh) and Tiptop(Tio),expressed in the progenitor cells of a developing eye disc,are also capable of repressing antennal/head epidermal selector genes. We have focused our attention on the potential role that Tsh and Tio have in repressing Cut(Ct), a key specification gene of the antenna and head epidermis. Our preliminary data indicate that both Tsh and Tio are capable of repressing ct when misexpressed in the antennal disc.This is strong evidence that they play a role in maintaining these tissues distinct fates.We are currently in the process of determining if both proteins impact ct expression directly or if the interaction is through other RD proteins, such as So and Eya. Tsh and Tio appear to function redundantly in the eye. We aim to determine the effect that removal of both genes has on the ability of the RD genes to continue to repress non-ocular selector genes in the eye. Our results will greatly enhance understanding of the molecular mechanisms underlying the specification and segregation of tissue fates.

766C

A screen for Notch-target enhancers that integrate homeotic factors and other signaling pathways. Elizabeth Stroebele, Timothy Fuqua, Ana Castro, Christian Noblett, Albert Erives. Department of Biology, University of Iowa, Iowa City, IA.

Many diverse signaling pathways are used during the development of a multicellular organism. These signaling pathways are integrated at enhancers and drive tissue-specific expression. We are interested in how Notch signaling information is integrated with other ubiquitous developmental signals at enhancers that drive a more limited tissue-specific expression. While there are about 20 well-studied Notch target enhancers, we have developed a computational pipeline to identify and study genus-wide conserved blocks that contain one or more Suppressor of Hairless [Su(H)] binding sites [5'-YGTGRGAA]. The conserved transcription factor, Su(H), is the downstream effector of the Notch signaling pathway, and at the DNA, Su(H) works as a repressor or as an inducible activator in conjunction with the Notch intercellular domain. Using three diverse *Drosophila* species we have we have identified 1,100 conserved candidate Notch-target enhancers. Of these candidate enhancers ~10% overlap with known enhancers, and 13% of those overlap with known Notch target enhancers. We have focused on testing candidates that have binding sites for transcription factors downstream of the Dpp signaling pathway (Mad and Brinker), the homeotic selector Apterous, and the pioneer transcription factor Zelda. In our screen, we discovered novel enhancers that drive distinct expression patterns in the developing wing imaginal disc and in the larval posterior midgut. A dorsal wing margin enhancer (DWME) at the *nab* locus drives a unique "Cleopatra-eye" expression pattern along the dorsal side of the wing imaginal disc margin. Interestingly, we found at least one other enhancer at the *hh/unk* locus, which drives a Cleopatra-eye pattern like the *nab* DWME, except this pattern is offset from it and is aligned around the presumptive wing hinge region. Both enhancers drive dual spot patterns on either side of Dpp expression. Both enhancers also share similar logic and are proving to be useful for understanding how their expression is restricted to specific areas of the wing disc.

767A

Combgap and Eyes absent balance regional identities during retinal development. Trevor Davis, Ilaria Rebay. Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, Chicago, IL.

Organogenesis requires the subdivision of unpatterned tissues into functionally distinct regions. One strategy to allocate cells to an

identity is for a gene regulatory network to execute the target developmental program while simultaneously repressing the competing program. Primordial *Drosophila* eye cells express Eyes absent (Eya) and Sine oculis (So), which physically interact to promote the transcription of retinal determination genes and to suppress the alternative head fate. How Eya-So activity is regulated to correctly apportion the eye and head cuticle is poorly understood. We hypothesize that the C2H2 zinc finger protein Combgap (Cg) represents an Eya-So co-factor that opposes Eya-So activity to balance competing eye and cuticle fates. In support of this model, lowering *cg* levels increases Eya's ability to block expression of the cuticle marker Cut in the presumptive retina and suppresses the loss of adult eye tissue in eya^{RNAi}-expressing animals. Furthermore, *eya* heterozygosity partially rescues the loss of presumptive head tissue in *cg* null eye-antennal discs, suggesting mutual antagonism between Eya and Cg in balancing eye-head cuticle regional identities. Mechanistically, Cg directly binds Eya and can participate in the Eya-So complex *in vitro*, and preliminary data imply that these interactions can impact regulation of Eya-So transcriptional targets. Together, this work suggests that Cg balances the assignment of eye and head fates by dampening Eya-So's promotion of retinal specification, by limiting Eya-So suppression of cuticle fate, and by promoting head development. Our current studies ask whether co-regulation of specific loci by Eya-So versus Cg-Eya-So complexes represents a mechanism for crosstalk between the competing genetic networks that designate regional identity in the eye disc.

768B

Role of *dve* in patterning and growth during eye development. N. Gogia[1*], A. Singh[1,2,3]. 1) DEPARTMENT OF BIOLOGY, UNIVERSITY OF DAYTON, DAYTON, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Centre for Tissue Regeneration & Engineering at Dayton (TREND), University of Dayton, Dayton, OH.

In multi-cellular organisms, axial patterning is required to generate three-dimensional organ from its primordia during organogenesis. *Drosophila* eye serves as an excellent model to study patterning and growth. In *Drosophila* eye, Dorso-ventral (DV) patterning is the first lineage restriction event in the developing eye. The early eye primordium begins with the default ventral fate on which the dorsal eye fate is established by expression of a GATA-1 transcription factor, pannier (pnr). We have identified *defective proventriculus (dve),* a K50 homeodomain gene as a novel dorsal gene that plays a crucial role in *Drosophila* eye development. We have found that *dve* acts downstream of *pannier (pnr)* in the developing eye. Loss-of-function phenotypes of both *pnr* and *dve* results in the dorsal eye enlargement. We will study role of *dve* and *pnr* in growth regulation during development. The results from these studies will be presented.

769C

Defining the role of Glass, a zinc finger transcription factor, in photoreceptor differentiation. Carolyn A. Morrison, Jessica E. Treisman. Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY.

Cell fate specification is achieved through the activities of master transcriptional regulators, which activate the developmental program necessary to establish a cell's identity. Glass (Gl) is a master transcriptional regulator required to establish the photoreceptor cell fate in the *Drosophila* eye. In *gl* mutants, eye progenitor cells correctly initiate neuronal differentiation but display defects in ommatidial organization and photoreceptor axon targeting, and ultimately fail to express photoreceptor-specific genes. To determine if Gl is sufficient to induce any or all aspects of photoreceptor development we generated a UAS-*gl* transgenic line and performed misexpression experiments in neuronal and non-neuronal cells. We show that Gl misexpression driven by *elav*-GAL4 in the embryonic nervous system is sufficient to induce the photoreceptor-specific gene *chaoptin* (*chp*) in a subset of neurons. We also show that misexpressing Gl in clones of non-neuronal cells in the third larval instar wing and leg discs is sufficient to induce Chp and that these cells are not converted to a neuronal identity. We plan to perform RNA-seq in order to identify which genes can be ectopically induced by Gl and in which cell types. A recent study identified 61 genes as likely direct transcriptional targets of Gl, characterized by both reduced expression in the eye disc of *gl* mutants, and the presence of predicted Gl binding motifs in their regulatory regions. Many of these genes are uncharacterized with no known function in eye development. We are performing an RNAi screen to elucidate their potential roles in photoreceptor development and axon targeting. Our preliminary results suggest a role for an LDL-CUB protein in R7 axon targeting and an EGF-like protein in eye development.

770A

A screen for negative regulators of damage-induced notum-to-wing transdetermination. Melanie I. Worley, Larissa Alexander, Iswar K. Hariharan. Molec & Cell Biol, Univ California, Berkeley, Berkeley, CA.

In *Drosophila*, the imaginal discs are capable of regenerating lost tissue after damage. However, damaged discs do not always regenerate the appropriate structures, and occasionally generate tissue appropriate for other imaginal discs or other parts of the same disc, a processes named "transdetermination" by Ernst Hadorn and colleagues. Transdetermination is the process by which cells with one determined state switch to a different determined state (reviewed by Hadorn, 1968). Low-level expression of *wingless* in leg imaginal discs has been shown to trigger leg-to-wing transdetermination (Maves and Schubiger, 1995) and several genes have been implemented in the process, including *dpp* and *bsk* (Johnston and Schubiger, 1996; Maves and Schubiger, 1998; Lee *et al.,* 2005; Klebes *et al.,* 2005; McClure and Schubiger, 2008; Ing *et al.,* 2013). However, the underling events that produce damage-induced transdetermination remain largely unknown, including the source and number of cells that change fate. We observed large differences in the frequency of notum-to-wing transdetermination following genetic ablation and regeneration (Smith-Bolton *et al.,* 2009) dependent on genetic background. In order to determine what genes function to prevent damage-induced transdetermination, we screened for deficiencies that dominantly enhance the frequency of notum-to-wing fate change. We screened over 200 deficiencies and found several loci that when deleted, in several independent lines, resulted in a high frequency of

transdetermination following ablation and regeneration. Further characterization of these loci will uncover the genes responsible and allow us to study the process of damage-induced cell fate changes.

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771B

A self-regulatory BMP signaling circuit drives sequential serosa and amnion specification in the scuttle fly *Megaselia abdita*. Chun Wai Kwan, Urs Schmidt-Ott. Organismal Biology and Anatomy, University of Chicago, Chicago, IL.

Self-regulatory signaling guides differential tissue specification in many contexts. Recent models emphasize the duration of signal exposure associated with signal refinement following "spatial overshoot" or changing competence of the responding tissue over time. Self-regulatory refinement of bone morphogenetic protein (BMP) signaling is also important for specifying dorsal ectoderm of Drosophila as amnioserosa tissue. Related fly species subdivide dorsal ectoderm in two extraembryonic tissues (serosa and amnion). Refinement and broadening of BMP signaling in correlation with early signs of serosa and amnion specification suggests a possible role of BMP-induced positive feedback in the specification of these tissues. We have found that the BMP target genes *dorsocross* (*doc*) and *hindsight* (*hnt*) are expressed throughout the extraembryonic zone in Megaselia and sustain a positive feedback loop whereby extracellular BMP signaling sequentially specifies the serosa and amnion. In *doc* and *hnt* RNAi embryos, amnion specification was disrupted. Double knockdown of both genes also interfered with serosa specification. Injection of *doc* or *hnt* mRNA induced amnion tissue ectopically in a BMP-dependent manner and perturbed BMP signaling in a manner indicative of positive feedback. Finally, early BMP signaling (before the late blastoderm cellularization stage) was required for both serosa and amnion development, but after this stage high BMP signaling was essential for amnion specification only. These data are consistent with the dynamic spatio-temporal pattern of BMP activity in Megaselia, first peaking at the site of serosa specification and then at the site of amnion specification. They are also consistent with published three-dimensional computational simulations of BMP signaling which, under enhanced feedback conditions, mimic the Megaselia pattern of BMP activity. We therefore propose a self-regulatory mechanism of sequential serosa and amnion specification in which early high BMP-signaling establishes the extraembryonic zone and specifies the serosa while subsequent high BMP-signaling "revisits" the amniotic fringe and finalizes amnion specification there.

772C

DV polarity establishment in the beetle Tribolium as revealed by RNAseq. Jeremy Lynch[1], Siegfried Roth[2]. 1) Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Institute for Developmental Biology University of Cologne Cologne, Germany.

The position of the oocyte nucleus late in oogenesis is strongly correlated to the future dorsal-ventral (DV) axis of the embryo in many insects. In Drosophila, an EGF signal from the oocyte to the follicle cells overlying the oocyte nucleus leads to the derepression of the transcription factor Mirror, which in turn represses *pipe*. Pipe activity establishes region in which Toll signaling will be active in the embryo. Our work has shown that while the EGF pathway's role in establishing DV polarity is highly conserved among insects, the downstream factors (e.g., pipe and mirror) are not. Since there are no additional clear candidate genes for converting EGF signaling to DV polarity, an unbiased approach in an alternative model organism is necessary. In the beetle Tribolium, the oocyte nucleus is very large and easily seen under low magnification. We have adapted the "embryo guillotine" to bisect Tribolium oocytes in late vittelogenesis into fragments that either do, or do not, contain the oocyte nucleus. These fragments were subjected to RNAseq, and by comparing transcript levels, we have identified strong candidates for transmitting EGF signaling, and DV patterning information, which are currently being validated by in situ hybridization and RNA interference. We have also identified a significant number of genes whose annotations indicate roles in regulating chromatin structure, completion of meiosis, and structure of the transcriptionally active Tribolium oocyte nucleus.

773A

Genome-wide measurement of Bicoid binding states at single positions along the AP axis. Colleen E Hannon, Shelby A Blythe, Eric F Wieschaus. Molecular Biology, Princeton University/HHMI, Princeton, NJ.

In order for embryonic development to proceed correctly and reproducibly, the expression of genes in individual cells must be coordinated with precision. In *Drosophila*, graded expression of the maternal transcription factor Bicoid (Bcd) provides positional information to pattern the anterior-posterior (AP) axis of the developing embryo. Bcd is known to bind hundreds of sites in the genome, thereby activating at least twenty target genes at different positions along the AP axis. One model to explain the positioning of Bcd target gene expression is through the binding affinity of their *cis*-regulatory elements. Genes in the anterior would have low affinity Bcd binding sites and could therefore only be activated by high Bcd concentrations, whereas genes expressed in more posterior positions would have higher affinity binding sites. While *in vitro* biochemistry using well-studied Bcd target gene enhancers has provided support for this model, the majority of the Bcd binding sites throughout the genome have not been extensively characterized. We have developed a method to use chromatin immunoprecipitation followed by high throughput sequencing to measure Bcd binding on a genome-wide scale in small samples of precisely staged embryos. To measure the Bcd binding state at 'single positions' along the AP axis, we have developed a series of transgenic lines that express consistent single, uniform concentrations of Bcd. We find that Bcd binding to enhancers that drive expression in anterior regions of the embryo is reduced in embryos expressing a low concentration of uniform Bcd, while binding to enhancers controlling posterior expression is maintained or increased. In embryos lacking posterior and terminal maternal patterning systems, despite dramatic differences in zygotic gene expression patterns, Bcd binding to the majority of its target enhancers is unchanged. These preliminary results support a model in which the positional information provided by Bcd is dependent upon its local concentration in the embryo, and is largely independent of inputs provided by other maternal patterning

systems.

774B

Background subtraction via nuclear Cactus increases the signal-to-noise ratio of the Dorsal gradient. Michael O'Connell, Gregory Reeves. Chemical & Biomolecular Engineering, North Carolina State University, Raleigh, NC.

Dorsoventral (DV) axis patterning in the early *Drosophila* embryo is controlled by transcription factor Dorsal (dl). A homologue of the mammalian transcription factor NF-kB, dl is inhibited from regulating gene expression by the inhibitor protein Cactus (Cact). Signaling through the Toll receptor along the ventral side of the embryo causes Cact degradation and nuclear uptake of unbound dl protein. Once dl becomes localized to the nucleus, it can promote or inhibit its target genes in a concentration-dependent fashion, including *Snail (sna)*, *Ventral Neuroblasts Deffective (vnd), Short Gastrulation (sog)*, and*Decapentaplegic (dpp)*. The working model of dl gradient formation has changed very little since its discovery in the late 1980s, despite tremendous advances in our quantitative understanding of the dynamics involved. We recently published a new model of dl/Cact dynamics that incorporates the trapping of both dl and Cact by the reforming, post-mitotic nuclear envelopes that shows excellent agreement with the results of live imaging published by Reeves et al. (2012). More importantly, our model suggested that Cact's presence in the nuclei increases the range of dl activity from that measured by fluorescence microscopy, explaining how dl can pattern genes on the lateral side of the embryo such as *sog* and *dpp*. Based on our analysis of noise in the model, we hypothesize that nuclear Cact acts as a background subtractor to increase the dl gradient's signal-to-noise ratio. This is especially important in regions where the gradient is seemingly too shallow for nuclei to reliably determine their proper location along the DV axis. Using our *in silico* model of gene expression, we can show how Cact minimizes the negative effects of extrinsic noise by increasing the relative difference between neighboring nuclei. Our modeling results suggest that performing a background subtraction operation may be one mechanism by which organisms maintain low-level control over concentration-dependent gene expression.

775C

A mathematical approach to predicting the mechanisms underlying the evolution of eggshell patterning by the TGF-alpha-like ligand Gurken. Nicole Pope[1], Nastassia Pouradier Duteil[1], Matthew Niepielko[1], Benedetto Picolli[1], Nir Yakoby[1,2]. 1) Computational and Integrative Biology, Rutgers University, Camden, NJ; 2) Biology Department, Rutgers University, Camden, NJ.

Morphology is highly diverse amongst *Drosophila* species; however the evolutionary mechanisms underlying morphological diversification are mostly unknown. The *Drosophila* eggshell provides an excellent system to study the morphological evolution of species. We found the dorsal ridge, a lumen-like structure along the dorsal-most side of the eggshells of numerous *Drosophila* species. The dorsal ridge is regulated by different distributions of the TGF-alpha-like ligand Gurken (GRK) in the egg chamber, the precursor of the mature eggshell. In dorsal ridge species, including *D. willistoni* and *D. nebulosa*, GRKs' localization extends halfway and all the way to posterior end of the egg chamber, respectively. This is in contrast to the midline restricted pattern of GRK in *D. melanogaster*, a species without a dorsal ridge. These spatial differences correspond to the final structure of the dorsal ridge on the flies' eggshells. The mechanism controlling different distributions of GRK is unknown. Considering that GRK is secreted from near the oocyte nucleus, and the position of the nucleus is dynamic in all species, beginning at the posterior end during early oogenesis and later anchored to the cortex of the oocyte, we developed a mathematical model to identify the parameters regulating differences among GRK gradients in these species. Our two-dimensional model integrates the movement of the oocyte nucleus, the diffusion of GRK in the perivitelline space, and the degradation rate of GRK. Numerous parameters, including GRK degradation rate and the speed of nuclear migration, are predicted to regulate different shapes of GRK gradient. These predictions are now tested experimentally. .

776A

From cells to pattern: A NetLogo model of Notch signaling. Elaine Reynolds[1], Ryan Himmelwright[1], Christopher Sanginiti[1], Jeffrey Pfaffmann[2]. 1) Neuroscience Program, Lafayette Col, Easton, PA; 2) Computer Science Dept, Lafayette Col, Easton PA.

The *Notch* (*N*) signaling pathway is involved in developmental patterning in diverse organisms. We have developed an Agent-Based model of this pathway that represents the major molecular components of the pathway. The model allows us to control the levels of these components, their transition from one state to another and their movement from the nucleus to the cell membrane and back. Most steps introduce randomness into the system using probabilities of events. Using this model, we are investigating the relationship between the stabilization of fate at the cellular level and the formation of pattern. A data logging mechanism captures an integer count of cells with zero *N* product (neurons) and the levels of *N* in all other cells at each time step and stores them as a number sequence that preserves cell position, linking cell behavior to pattern dynamics. The system exhibits oscillations before setting into a stable pattern. Using a plot of neuron count vs. time, we can determine a stabilization time using a measure of deviation, the final count of neurons, and an assessment of pattern that can be compared to an optimal pattern. To quantitate pattern, we use a modification of Hamming distance that measures the amount of dis-similarity at successive spatial positions within the model. Within a wide set of initial parameters, our current model can accurately reproduce the rosette pattern of neurons and skin cells. We have explored the roles of initial transcriptional rates, the endocytic processing of *Dl*, transport of *N* after cleavage, and the regulation of transcription by *N*. Results indicate that initial transcription rate can be varied and a stable pattern is still obtained. There is a sweet spot where stabilization rates are long enough to allow a proper pattern to form, similar to the ideas of landscape first proposed by Waddington (1959). *Dl* and *N* processing parameters shift the optimums in this landscape, but overall do not disrupt the formation of pattern. However, the model only achieves the proper pattern and number of neurons when there is transcriptional feedback to *N* and *Dl* expression.

777B

Evolution in silico of genes with multiple regulatory modules: gap genes vs pair-rule genes. Alexander Spirov[1], David Holloway[2]. 1) Cntr Excellence in Wireless & Information Technol, State Univ New York, Stony Brook, NY; 2) Mathematics Department, British Columbia Institute of Technology, Burnaby, B.C., Canada.

Developmental genes have extensive cis-regulatory regions which control their expression. These regions are organized in a modular manner, with different modules controlling expression at different times and locations. Both how modularity evolved and what function it serves are open questions. A particular area of interest in evolutionary development (evo-devo) is the correspondence between gene regulatory sequences on the DNA (cis-regulatory modules, CRMs) and the spatial expression of the genes. We use in silico evolution to investigate the incorporation of new CRMs into the genome. Simulations allow us to characterize different cases of CRM to spatial pattern correspondence. Many of these cases are seen in biological examples; our simulations indicate relative advantages of the different scenarios. We find that, in the absence of specific constraints on the CRM-pattern correspondence, CRMs controlling multiple spatial domains tend to evolve very quickly (multi-CRM/multi-domain correspondence). We find that the CRM-domain correspondence seen in Drosophila evolves with a high probability in our model, supporting the biological relevance of the approach. The partial redundancy resulting from multi-CRM control may confer some biological robustness against corruption of regulatory sequences. Genes constrained to a one-to-one CRM-pattern domain correspondence evolve more slowly. Of these, systems in which pattern domains appear in a particular order in evolution, as in insect segmentation mechanisms, take the longest time in in silico evolutionary searches. For biological cases of this type, it is likely that other selective advantages outweigh the time costs.

778C

Robust patterning of the dorsal-ventral axis in the *Drosophila melanogaster* embryo. Sophia Carrell, Alexander Thomas, Jeramey Friedman, Gregory Reeves. Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

During nuclear cycles 10-14, two conserved signaling pathways act to pattern the DV axis of the *Drosophila* embryo: the Dorsal pathway and the Dpp pathway. On the ventral side of the embryo, the transcription factor Dorsal, homologous to NF-kB, triggers expression of the genes that initiate the DV pattern. Dorsal is present in a nuclear gradient, with the highest concentration at the ventral midline and a steady decay to about 40% of the embryo's circumference. Beyond the expression domain of Dorsal, the dorsal side of the embryo is patterned by BMP signaling via the ligand Dpp. Dpp signals through the receptor Thickveins (Tkv), phosphorylating the receptor Smad protein MAD. Two pMAD molecules bind to the co-Smad Medea; this heterotrimer then enters the nuclei to facilitate expression of target genes. Together, the nuclear gradient of Dorsal and BMP signaling work to pattern the DV axis of developing Drosophila embryos. Because robustness of tissue patterning is essential for proper embryonic development, regulatory loops must exist to ensure correct placement of target genes in the face of perturbed conditions. As an example, embryos with a half dose of Dorsal protein survive to adulthood. Feedback through the Dpp signaling network is a prime candidate for enhancing the robustness of Dorsal patterning of DV gene expression. We investigated the effects on the shape of the Dorsal gradient of overexpression of MAD and Medea individually, as well as expression of constitutively active and dominant negative forms of the receptor Tkv. We found that in each of these cases, the Dorsal gradient was statistically wider than wild-type, indicating interactions between the two signaling pathways, consistent with the research of other groups. These interactions between the NF-kB and BMP signaling pathways may be necessary to ensure robust gene expression in the developing *Drosophila* embryo.

779A

The Instructive Epidermis: The Role of JNK Organizing Centers. Molly Jud, Anthea Letsou. Human Genetics, University of Utah, Salt Lake City, UT.

During development of multicellular organisms, cells within seemingly uniform cellular fields are marked for unique fates. Thus, while two cells may appear physically identical, one may be molecularly distinct from its neighbor. Understanding how differential signaling domains are partitioned within cell fields is important for appreciating how morphological processes are coordinated and how tissues are specified during development. We have identified several previously unrecognized JNK signaling-centers within the epidermis of the developing Drosophila embryo through a genetic analysis of mutations in the JNK signaling antagonists Raw, Ribbon (Rib), and Puckered (Puc). Here we show that *raw*, *rib*, and *puc* mutants exhibit shared loss-of-function phenotypes in: (1) the embryonic epidermis (dorsal closure defects and hypotrophy of ventral denticle belts), and (2) the peripheral nervous system (PNS; fasciculation defects and thickened and misguided axons), suggesting contemporaneous roles for JNK signaling in patterning both tissues. We show that early in development (4-8 hours AEL), JNK signaling is activated uniformly in the dorso-lateral embryonic epidermis. By mid-embryogenesis, this broad signaling domain is replaced by a JNK signaling domain that: (1) is confined to leading edge epidermal cells, (2) is maintained along the dorsal-ventral axis, and (3) functions as an organizing center for epidermal closure and mesodermal heart development. We also show that JNK signaling is differentially activated in single cells of the lateral epidermis lying directly above the PNS and that JNK signaling is required for the nervous system defects observed in both *raw* and *rib* mutants, suggesting that epidermal cues serve to pattern the underlying PNS. In understanding the mechanism of action of multiple JNK signaling antagonists, we have found that the epidermis itself plays an instructive role during development. This instructive epidermis informs our understanding of cellular coordination, communication, and tissue specification and provides tools for further investigation into how such processes effect development and disease on an organismal scale.

780B

Taranis buffers regenerating tissue from fate changes induced by the wound response in *Drosophila*. Keaton Schuster, Rachel Smith-Bolton. Cell & Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

Regenerating tissue must replace lost structures with cells of the proper identity and function. How regenerating tissue establishes or maintains correct cell fates during regrowth is an open question. To identify genes that are important for patterning and cell fate after tissue damage, we use genetic tools to ablate tissue in the wing imaginal discs of *Drosophila* to screen for and characterize genes required for regeneration. We have identified a gene, *taranis*, which is essential for maintaining proper cell fate in damaged and regenerating wing imaginal discs, but is dispensable for normal wing development. In regenerating tissue with reduced levels of Taranis, expression of the posterior selector gene *engrailed* is silenced through an autoregulatory silencing mechanism that requires the PRC1 component*polyhomeotic*, resulting in a transformation of posterior tissue into anterior tissue. An essential component of the wound response, JNK signaling, induces this misregulation of *engrailed* expression. Thus, Taranis functions to stabilize *engrailed* expression in cells experiencing high levels of JNK signaling. We are examining whether Taranis acts on *engrailed* directly, because it does not regulate JNK activity and is not, in turn, regulated by JNK signaling. Through this work we will clarify how *taranis* buffers regenerating tissue from deleterious side effects of wound healing and regeneration.

781C

Identification of the specific *de2f1* transcript that is required for the formation of imaginal discs. Mary-Rose Bradley-Gill, Nam-Sung Moon. Biology, McGill, Montreal, Quebec, Canada.

E2F family transcription factors are evolutionarily conserved regulators of the cell cycle that are required for animal development in most species. E2F proteins can be divided into two groups based on their ability to either activate or repress transcription. In *Drosophila*, there is only one "activator" E2F, dE2F1, which provides all the pro-proliferative activity of E2F during development. Interestingly, the*de2f1* gene can be transcribed from multiple promoters resulting in six alternate transcripts that differ only in their 5'UTR sequence. Despite this complexity, not much is known about how dE2F1 expression is regulated at the level of transcription. To determine the purpose of having different transcription start sites, we examined transcript specific *de2f1* mutants. By analyzing different insertional mutants and generating a deletion mutant, we identified a specific *de2f1* transcript that is necessary for the proper development of imaginal discs and larval brain. Genomic deletion covering exon 1 of the specific transcript results in larvae that have small imaginal discs and brains. Importantly, the sizes of the fat body and salivary glands are relatively normal, indicating that the effect of deletion is specific to mitotically dividing cells at the larval stage. Our study demonstrates that different *de2f1* promoters are used to express dE2F1 in response to specific developmental signals and suggests that the transcription of *de2f1* is highly regulated.

782A

Genome wide identification of Smad2 target genes mediating TGFβ function during *Drosophila* imaginal development. Covadonga F. Hevia, Jose F. de Celis. Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain.

Activin/TGFβ signaling regulates a broad range of cellular processes during the development of all metazoa. In *Drosophila*, we have previously proposed that the pathway primarily promotes cell division during wing development, but the genes mediating this activity are still unknown. Moreover, direct target genes of Smad2, the transcription factor that functions as the final effector of the pathway, have not been systematically characterized in *Drosophila*. To identify target genes of Smad2, we performed expression microarrays in *Smad2* mutant conditions and ChIP-on-Chip assays with Smad2. We identified a group of genes that are subjected to Smad2 transcriptional regulation in the wing disc and whose regulatory regions are bound by Smad2, so they could be direct targets of the pathway. These genes are mostly expressed in a generalized manner in the wing disc, mirroring the spatial activation of the pathway. In *Smad2* mutant backgrounds, the general behavior of candidate target genes is to undergo up-regulation in a Smad2-dependent manner, indicating that Smad2 most likely functions as a transcriptional activator. We also examined the functional requirements of the candidate genes by RNA interference, and identified those required to control wing size. In this manner, our study reveals a group of genes that might be mediating the function of the pathway in the control of cell proliferation of the wing disc.

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783B

The *triia-s-2* gene encodes a male germ-line specific homolog of the small subunit of the TFIIA General Transcription Factor. Mark Hiller, Ashley Cowan, Leah Hirschman, Maura Coughlin. Dept of Biological Science, Goucher College, Baltimore, MD.

Developmentally regulated transcription brings about cellular differentiation in multicellular animals. Spermatogenesis depends on robust and dynamic transcriptional regulation, and we have been investigating the role of tissue-specific forms of the General Transcription Factors in spermatid differentiation. The General Transcription Factors are multi-protein complexes and essential for transcription in Eukaryotes. In *Drosophila melanogaster*, several homologs of more generally expressed subunits are expressed only in the male germline. TFIID is comprised of TBP (TATA-binding protein) and up to fourteen TAFs (TBP-associated factors), and several homologs of TAF's are testis-specific. In *D. melanogaster* there are also two homologs of TBP, TRF1 and TRF2, that are widely expressed in the fly including the male germ-line. Another General Transcription Factor, TFIIA, physically associates with TFIID at promoters and helps position RNA Polymerase at promoters of transcribed genes. TFIIA consists of three protein subunits. A single gene, *tfiia-l*, encodes a single polypeptide that is protolytically cleaved to form two proteins of 30 kD and a 20 kD. A separate gene, *tfiia-s*, encodes the small subunit, a 14 kD protein. We have shown that the gene *tfiia-s-2* (CG11639) encodes a male germ-line-specific homolog of the

14kD subunit and that *tfiia-s-2* encodes two different messages. Both TFIIA-S-2 proteins associate with TFIIA-L *in vitro*, implying that three different forms of TFIIA are present in *D. melanogaster* testes. These forms of TFIIA may interact with either TAFs or the testis-specific TAFs to regulate gene expression in the testis. We are characterizing the ability of TFIIA-S-2 containing complexes to physically associate with subunits of TFIID, including TBP and the TBP associated factors (TAFs). In order to probe the function of the testis-specific TFIIA subunit we are creating a loss of function allele. .

784C

The dosage compensation protein CLAMP is involved in non sex-specific histone transcript misprocessing. Leila Rieder[1], Anna Zeidman[1], Katy Curry[2], Bob Duronio[2], Erica Larschan[1]. 1) Molecular Biology, Cellular Biology and Biochemistry, Brown University, Providence, RI; 2) Department of Genetics, University of North Carolina, Chapel Hill.

CLAMP (Chromatin Linked Adapter for MSL Proteins) is a zinc-finger transcription factor involved in recruiting Male Specific Lethal (MSL) complex to the male X chromosome during dosage compensation. Yet CLAMP, which binds to GA-rich sequences, localizes throughout the genome in both males and females, and is essential for adult development in both sexes. CLAMP appears to have an essential non sex-specific function, in addition to its role in male-specific dosage compensation. One function of CLAMP may be at the Histone Locus Body (HLB). The histone locus on chromosome 2L contains ~100 repeats of the core histone genes. Factors involved in transcriptional control and unique histone transcript processing are recruited to this locus and form the nuclear body known as the HLB. CLAMP co-localizes with markers of the HLB in both sexes and localizes to the minimal 300bp region required for HLB formation, which contains two GA-rich motifs. Depletion of CLAMP *in vivo* results in a histone transcript misprocessing phenotype.
We present evidence that CLAMP localization to the HLB is required for proper histone transcript processing and situate CLAMP in a cell cycle model with other HLB factors.

785A

Molecular Genetic Mechanism for Development of The Small Intestine in Drosophila Hindgut. Sarder Uddin[1], Ryutaro Murakami[2]. 1) Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh; 2) Graduate School of Medicine, Yamaguchi University, Japan.

An odd family gene drumstick (drm) encodes a zinc finger protein, and is necessary for development of the small intestine, an anterior domain of the ectodermal hindgut of Drosophila . However, mechanisms that specify the small intestine, as well as gene regulatory pathways leading to transcriptional activation of drm, are still unclear. We found that drm is expressed in two different tissues abutting anterior end of the hindgut primordium, that is, the posterior-most region of the midgut (endoderm) and basal portion of the Malpighian tubules. A small intestine marker gene, unpaired (upd), begins to be expressed at the anterior-most region of the hindgut primordium that abuts the basal portion of Malpighian tubules, and the upd-positive region expands, resulting in a short tube during stages 11-13. Small intestine develops in both of the mutant embryos, srp and Kr, that lack the drm-positive midgut or Malpighian tubules, respectively, while small intestine fails to develop in the Kr srp double-mutant embryos that lack both of the drm-positive tissues. These results demonstrate that drm expressed in the abutting tissues cell-non-autonomously induces development of the small intestine in the hindgut primordium, probably by deploying some extracellular signaling factor. drm expression in the posterior gut region disappears and the small intestine fails to form in tailless (tll) mutant embryos, while over-expression of tll causes expansion of drm expression throughout the midgut, inducing longer small intestine. These results indicate that drm is activated under the control of tll, and triggers development of the small intestine cell-non-autonomously through some extracellular signaling.

786B

A Role for *Valois* and *Piwi* in the Maternal to Zygotic Transition. Shelby Blythe, Eric Wieschaus. Molecular Biology, Princeton University/HHMI, Princeton, NJ.

The midblastula transition (MBT) represents the coordinated remodeling of both the cell cycle and transcriptional activity as embryos transit from maternal to zygotic control of development. The temporal control of the MBT derives in large part from a poorly understood mechanism that monitors the embryonic nucleo-cytoplasmic (N:C) ratio. Upon reaching a threshold N:C ratio, nascent zygotic gene activation (ZGA) drives cell cycle remodeling, initially by activating an ATR/Chk1-dependent DNA replication checkpoint. Disruption of ZGA can therefore lead to changes in the number of pre-MBT mitoses. We report here our analysis of *valois* mutant embryos, which undergo one additional, Chk2-dependent, pre-MBT mitosis with delayed ZGA. Valois is the *Drosophila* homolog of MEP50, an essential cofactor for the arginine methyltransferase Prmt5/Capsuleen, which is involved in spliceosome assembly, piRNA biogenesis, and posttranslational modification of histone proteins. Valois is necessary for nuclear localization of Piwi during syncytial development, and *piwi* germline clone embryos likewise undergo an additional syncytial mitosis. We present evidence for a role of *valois* and *piwi* in triggering the DNA replication checkpoint at the MBT via regulation of the timing of ZGA.

787C

The role of SAGA subunits for its genome-wide recruitment to chromatin. G. Dialynas, S. Abmayr, A. Peak, A. Perera, C. Seidel, J. Workman. Stowers Institute For Medical Research, Kansas City , MO.

In the native chromatin environment, the packaging of eukaryotic DNA into nucleosomal arrays constitutes a major obstacle to DNA-templated cellular processes, such as transcription, replication and DNA repair. The discovery of large multi-protein complexes that facilitate chromatin remodeling in order to grant access to DNA and recruitment of downstream effectors has led to new insights into the regulation of transcription. The SAGA complex, a major transcriptional co-activator identified and purified from yeast, is required for

efficient expression of many inducible genes and is recruited to promoters of target genes through the interaction with the sequence-specific DNA-binding transcription. Eighteen to 20 protein subunits comprise the SAGA complex, which is evolutionarily conserved in every organism through humans. The general structure and function of the five SAGA modules appears to be conserved from yeast to humans. Nevertheless, the SAGA modular composition may be further refined, and there may be differences in module composition and function that are specific to metazoans. The expression of SAGA-occupied genes in a specific tissue may not require all SAGA modules; individual modules may play unique roles in different tissues or cell types. In order to understand the importance of specific SAGA subunits and activities in each tissue, we use the fruit fly model system to examine their roles in the recruitment of SAGA to promoters on a genome-wide level. To accomplish this we knock down various SAGA subunits using the Gal4/UAS system and RNAi tools. Our morphological studies revealed that SAGA subunits exhibit distinct banding patterns, localizing at multiple genomic sites on polytene chromosomes from wild-type flies, while the knockdown of a SAGA subunit disrupts the recruitment of other subunits. These data show that our approach of tissue specific knockdown of SAGA subunits using the Gal4/UAS system in Drosophila is successful allowing us to examine these effects in more detail and depth at a genome wide level.

788A

***Notch* alleles encoding variant polyQ tracts disrupt anterior-posterior patterning in *Drosophila* embryos. Albert Erives**, Clinton Rice, Danielle Beekman, Rachel Harney, Megan Bowman, Sarah Coe, Liping Liu, Megan Tobias, Mark Vaske. Department of Biology, University of Iowa, Iowa City, IA.

Notch (N) signaling is involved in numerous developmental processes and decisions throughout Metazoa; and while many protein interactions in the N pathway have been well-studied in *Drosophila*, novel functions continue to be discovered. One region in the N intracellular domain (N_{ICD}) that has received little study is a polyglutamine tract of 30 Q's interrupted by a single histidine (pQ = "Q_{31}"). We identified *Drosophila* Genetic Reference Panel (DGRP) lines carrying variant N_{ICD} pQ tract alleles, including two of 23 and 35 residues in length. Outcrossed Q_{23} and Q_{35} lines show increased failure to hatch compared to the Q_{31} lines, and temperature seems to affect hatching rate of the Q_{23} line, but not the Q_{35} line. Adult phenotypes (doubled macrochaetae and A/P patterning defects) also persisted after outcrossing. No A/P phenotypes were noted for the stock Q_{35} line, but after outcrossing, 50% of females showed A/P phenotypes similar to the Q_{23} flies, suggesting the possibility that some established DGRP stocks also contain modifying suppressors. We investigated a number of unlinked loci encoding N interacting factors with pQ tracts and found that some of these were characterized by pQ variant alleles in linkage disequilibrium with the *N* locus. A/P patterning defects were also observed when N target gene expression was examined in early embryos. The normal pattern of *rhomboid* expression, a key source of A/P and D/V modulated EGF signaling, is altered in the posterior domains of many Q_{23} embryos. Interestingly, we found that different mutations in the *CG9281* gene, which encodes an embryonic ABCF2 chaperone that interacts with N_{ICD}, also result in similar A/P patterning defects, suggesting the existence of chaperone-mediated autophagy in some embryonic contexts of N processing. Altogether, these results suggest that N processing in the early fly embryo is important for A/P patterning, as observed in other arthropods (*e.g.*, spiders) and vertebrates (*i.e.*, somitogenesis and neural segmentation). It also suggests a specific role for the polyQ tract in the embryonic processing or perdurance of N_{ICD}.

789B

COMPASS-like coactivator complex regulation of the *bantam* miRNA enhancer. David J Ford[1], Claudia B Zraly[2], John S Perez[2], Andrew K Dingwall[1,2,3]. 1) Molecular Biology/Biochemistry Program; 2) Oncology Research Institute; 3) Department of Pathology. Loyola University Chicago-Stritch School of Medicine, Maywood, IL.

The evolutionarily conserved COMPASS-like complexes function as coactivators of nuclear receptors and other transcription factors that control multiple developmental signaling pathways in all higher eukaryotes. The Drosophila Cmi(Lpt)/Trr COMPASS-like complex monomethylates H3K4, a histone modification found in chromatin that is generally associated with transcription enhancer elements. The genes encoding the Cmi and Trr core subunits arose from a single common ancestor. In Drosophila this ancestral gene underwent a fission event, separating into two independent genes, *cara mitad (cmi)*and *trithorax-related (trr)*. Cmi contains several plant homeodomain Zn-fingers (PHDf) that bind histones and serve as epigenetic readers, while Trr provides histone methyltransferase activity. In vertebrates the Cmi and Trr homologs are single proteins MLL2/4 and its close paralog MLL3, which have gained recent prominence as frequent targets of mutation in a wide variety of cancers as well as developmental and intellectual disorders. The Cmi/Trr COMPASS-like complex functions as a coactivator of EcR/USP during hormone dependent gene activation and it regulates the TGFβ and Hippo developmental signaling pathways that converge on *bantam*, an anti-apoptotic micro-RNA produced from a long non-coding RNA (lncRNA) that contributes to the control of insulin signaling and growth. ChIP analyses reveal that the Cmi/Trr complex localizes to tissue-specific *bantam* enhancers and knock-down of *trr* and *cmi* result in the up-regulation of *bantam* expression, thereby functioning as negative regulators of transcription. We have used *bantam* enhancer-reporters with tissue-specific knockdown of components of the Cmi/Trr complex combined with epigenome analyses to determine the epigenetic regulatory functions of the complex in controlling *bantam* miRNA expression. This investigation will provide unique insights into the mechanism of the conserved COMPASS-like complex in establishing transcription enhancer identity and help to elucidate how loss of the complex contributes to disease.

790C

***Akirin*-Mediated Gene Regulation during cardiac development. Austin M. Howard**, Scott J. Nowak. Department of Biology and Physics, Kennesaw State University, Kennesaw, GA.

The highly conserved nuclear protein Akirin was previously identified as a cofactor that modulates Twist transcription factor activity

during muscle development in *D. melanogaster*. Akirin mediates an interaction between the Twist transcription factor and the multisubunit Brahma SWI/SNF-class chromatin remodeling complex at control elements of the *Dmef2* locus to maintain optimal myogenic gene expression levels. Therefore Akirin represents a class of novel secondary cofactors that work with transcription machinery to link transcription factor output with chromatin remodeling machinery to facilitate gene expression . Previous work establishes that Twist and Akirin also interact at Twist-responsive control elements of the *tinman* gene, which regulates formation and development of the *Drosophila*dorsal vessel. Similar to other Twist-regulated loci, Akirin appears to positively regulate *tinman* expression and affect development of cardiomyocytes. Together these results uncover a potential new method of regulation for the *tinman* locus during cardiac development. .

791A

The transcriptional network of rhabdomeric photoreceptor differentiation. Xulong Liang, Simpla Mahato, Savanna Brewski, Andrew Zelhof. Biology, Indiana University Bloomington, Bloomington, IN 47405, IN.

Photoreceptor cells are required for light-dependent physiological functions, visual or non-visual. In most Protostomia, vision is achieved by one particular photoreceptor type, the rhabdomeric photoreceptor. The rhabdomeric photoreceptor is characterized by the transformation of the apical membrane into a structure containing thousands of tightly packed microvilli responsible for housing the phototransduction machinery, rhabdomeric opsins and their downstream signaling proteins. To date, there is considerable knowledge of molecular mechanism of retinal determination and photoreceptor specification, but very little effort has been made to decipher how rhabdomeric terminal differentiation, in terms of morphogenesis and function, is regulated on the molecular level. Here we will present our genetic screens and results to identify the regulatory network required for the transcriptional control of photoreceptor terminal differentiation as well as other molecules responsible for the morphogenesis of the rhabdomere. .

792B

Regulation of mitochondrial function by the transcriptional regulator KDM5. Xingyin Liu, Julie Secombe. Genetics, Albert Einstein Med College, Bronx, NY.

Drosophila KDM5 (also known as Lid) and its four mammalian homologs, KDM5A, KDM5B, KDM5C and KDM5D, are multi-domain transcriptional regulators. In humans, KDM5A or KDM5B overexpression is associated with breast, gastric and prostate cancers, and mutations in KDM5C are found in patients with intellectual disability. However, a confounding factor to the analysis of the four mammalian KDM5 paralogs is their functional redundancy. In contrast, Drosophila has a single KDM5 protein, providing an ideal system to delineate the function of this family of proteins. To identify KDM5-regulated genes in the Drosophila adult, we carried out RNA-seq from wildtype and *kdm5* hypomorphic mutant flies. These analyses revealed that 2006 genes were up-regulated and 2226 genes were down-regulated 1.5-fold or more in *kdm5* mutants. Interestingly, KEGG pathway analyses of up-regulated transcripts revealed enrichment for genes required for mitochondria oxidative phosphorylation pathway. While increased levels of respiration components might be expected to increased energy production, we find that *kdm5* mutants have decreased cellular ATP levels. This may be explained in part by the down-regulation of genes required to maintain mitochondrial membrane potential in *kdm5* mutants. Indeed, the thoracic flight muscles of *kdm5* mutant flies have fewer mitochondria that are deformed. Because mitochondria defects have been proposed to contribute to both cancer and cognitive disorders, we believe that our analyses has significant implications for understanding the link between dysregulation of KDM5 family proteins and human disease. Currently, we are performing the combined KDM5 Chip-seq and RNA-seq analysis to reveal how KDM5 regulate mitochondrial function.

793C

Mapping the transcriptional network regulated by the conserved factor Grainyhead during embryonic development. Markus Nevil, Eliana Bondra, Melissa Harrison. Department of Biomolecular Chemistry, University of Wisconsin - Madison, Madison, WI.

The highly conserved Grainyhead (GRH) transcription factor family has essential functions during development and when mutated leads to severe neural tube closure defects in mammals. Drosophila contain a single *grh* gene, the founding member of this family that is conserved from fungi to humans. Through the regulation of gene expression, GRH controls a variety of processes in flies ranging from neuroblast differentiation to epithelial morphogenesis, and embryos lacking GRH die late during embryogenesis. Nonetheless, the transcriptional network regulated by GRH and how GRH binding and gene expression changes over the course of development remains to be fully elucidated. Furthermore, data suggest that GRH can function as both a transcriptional activator and repressor but it is currently unclear how it performs these two functions and whether its role in transcriptional regulation changes over development. We have used a combination of ChIP-seq and RNA-seq over the course of embryonic development to define the transcriptional network regulated by GRH. By analyzing GRH binding sites at four developmental time points, we determined that GRH binds thousands of sites and these binding sites remain relatively stable over embryonic development. By performing RNA-seq on embryos lacking either maternal or zygotic GRH, we have defined those sets of genes that are activated and repressed by GRH and how they change over development. Initial analysis suggests that late in embryonic development, GRH is primarily acting as a transcriptional activator. We will present further analyses of these data and discuss our model for how this essential transcription factor functions during development. .

794A

Klumpfuss/Wilms' tumor suppressor controls stochastic gene expression in the fly eye. Caitlin C Anderson[1], Ben Mormann[2], Daniel Vasiliauskas[2], James Taylor[1], Robert Johnston[1]. 1) Biology, Johns Hopkins University, Baltimore, MD; 2) Biology, New York University, New York, NY.

Stochastic gene expression is important for several developmental processes, including proper immune cell fate determination, stem cell differentiation, and sensory receptor diversification. The random mosaic of color vision photoreceptors within the *Drosophila Melanogaster* retina is controlled by the stochastic expression of the transcription factor Spineless (Ss). *ss* expression is highly variable between wild-derived fly lines. Using GWAS to identify genetic causes of this variation, I discovered a single SNP that was highly associated with a low frequency of *ss* expression. Remarkably, this SNP is located within the *spineless* locus as well as a putative binding site for the transcription factor **Klumpfuss** (**Klu**), the fly homolog of Wilms' Tumor Suppressor. Klu is expressed in all R7 cells early in eye development, suggesting that it may play a regulatory role. Investigation of *klu* mutants and ectopic expression revealed that Klu levels inversely affect *ss* expression: as Klu levels increase, the frequency of stochastic ss expression decreases, and vice versa. Based on this inverse relationship between Klu levels and ss expression frequency, we hypothesize that Klu acts as a repressive rheostat to control ss expression. Moreover, we predict that the low ss SNP increases Klu binding causing repression of ss. These data suggest a novel mechanism whereby a tumor suppressor protein acts as a rheostat to control stochastic gene expression.

795B

Identification of the Lola Transcriptional Targets during Embryonic Development. Edwin Chaharbakhshi, Christopher Lenkeit, Jennifer Jemc, Safiyah Elahi. Loyola University Chicago, Chicago, IL.

Cell-cell interactions are necessary for the coalescence of cells into functional organs during development. In order to develop a deeper understanding of the basis by which various cell types interact during embryonic development, we must discover the nature by which genes vital for development are regulated. In particular, mutations in the longitudinals lacking (*lola*) gene, which codes for a transcription factor with at least 26 known isoforms, result in observable developmental defects throughout the nervous system and the gonads. Several studies have suggested that *lola* is responsible for altered expression of numerous genes during development through transcriptional regulation. Data collected in previous studies provides us with predicted isoform-specific Lola DNA binding domains and one identified direct target: the copia retrotransposon. Additionally, microarray data concerning *lola* mutants have suggested several of Lola's potential binding targets involving axonal growth, yet targets have not yet been confirmed involving gonad morphogenesis. By compiling the data from previous studies with database sequences of potential binding sites of the Lola transcription factor, we want to identify additional genes regulated by select Lola isoforms expressed in the gonad and its binding sites. In order to test the data generated through this compilation, we generated a list of potential direct targets. We are performing chromatin immunoprecipitation (ChIP) and quantitative PCR (qPCR) in order to confirm which genes Lola regulates and to further characterize the DNA binding consensus sequences for several Lola isoforms. .

796C

Identification of Transcriptional Targets of Longitudinals Lacking. Christopher P Lenkeit, Jennifer Jemc, Edwin Chaharbahkshi. Loyola University Chicago, Chicago, IL.

In order to form functional tissues, multiple cell types must be specified and migrate to the appropriate location and interact properly. Any disruptions during this process will result in failure of gonad development. At different times the transcription of genes must be promoted or inhibited by various transcription factors in order to maintain the correct concentration of proteins. My research will focus on the transcription factor longitudinals lacking (Lola), which is a transcriptional repressor. Previous research has confirmed that the protein is expressed in both *Drosophila* somatic gonadal precursor cells (SGPs) and germ cells (GCs), and is required for gonad morphogenesis. Lola is also a Broad complex, Tramtrack, Bric-a-Brac (BTB) transcription factor and is therefore likely to interact with other BTB domain containing proteins to regulate transcription. Lola undergoes extensive splicing, yielding 26 different isoforms, making it difficult to ascertain specific functions for Lola in different tissues. Recent publications have confirmed Lola R/G's role in the SGPs, but the role of other isoforms predicted to be expressed in the SGPs and GCs remains to be explored. I have utilized information from other sources including, predicted Lola binding motifs, microarray studies, and modENCODE data, which has allowed me to form a list of potential genes downstream of Lola. Chromatin immunoprecipiation and quantitative PCR is being used to confirm potential Lola targets. These targets will give insight into how Lola regulates tissue morphogenesis during development.

797A

Instability element of retinoblastoma protein Rbf1 regulates apoptotic and developmental phenotypes. Rima Mouawad[1], Jared Elenbaas[2], Yiliang Wei[2], Sandhya Payankaulam[2], David Arnosti[1,2]. 1) Cell and Molecular Biology, Michigan State University, East lansing, MI; 2) Biochemistry and Molecular Biology, Michigan State University, East lansing, MI.

Retinoblastoma (Rb), a tumor corepressor, is involved in major cellular processes including proliferation, differentiation and apoptosis, and is found to be deregulated in a broad range of human cancers. Rb activity is regulated by cyclin/cdk-mediated phosphorylation and its turnover is through the ubiquitin-proteasome system. Previously, we identified an instability element (IE) in the C-terminus of RBF1, the*Drosophila* Rb homolog, which regulates RBF1 stability as well as its repression activity. We find that lesions in the IE have a gene-specific effect. Strikingly, overexpression of RBF1-ΔIE in wing imaginal discs resulted in larger wings, while RBF1-K774A (mutant lysine within IE) overexpression resulted in small defective wings. To obtain a molecular understanding of these phenotypes, we performed qRT-PCR and acridine orange staining to measure apoptosis. Interestingly, RBF1-ΔIE mutant resulted in repression of apoptosis in contrast to K774A mutant which resulted in induction of apoptosis. This indicates that lesions in IE have neomorphic functions and do not reflect an Rbf1 loss of function which is frequently associated with cancer. We are conducting RNA-seq studies to understand the role of these Rbf1 isoforms on global gene regulation. We propose that IE is required for repression of certain classes of genes based on distinct interactions with E2F transcription factors, and K774 residue is critical for normal RBF1 function.

798B

Transcriptional regulation of cell polarity determinants by the Retinoblastoma tumor suppressor protein (Rbf1) in *Drosophila melanogaster*. Sandhya Payankaulam[1], Kelvin Yeung[2], Helen McNeill[2], William Henry[1], David Arnosti[1]. 1) Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48823, USA East Lansing, MI; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Room 881, Toronto, Ontario M5G1X5, Canada.

A comprehensive profiling for physical targets of the fly Rbf1, using genome wide ChIP sequencing technique revealed that Rbf1 occupies genes encoding components of Insulin, Hippo, Wnt and the Jak/Stat signaling pathways. Intriguingly, Rbf1 was also found to target core components of the less understood but highly conserved apical-basal and planar cell polarity (PCP) pathways. To define a possible role for Rbf1 in polarity, we reduced the level of Rbf1 in the developing *Drosophila* wing imaginal discs through RNAi and tested for PCP pathway effects in the emerging adult fly wings. Reducing the level of Rbf1induced polarity defects in *Drosophila* tissues. Furthermore, we observed altered mRNA levels of key polarity genes such as *apkc*, *par6*, *vang*, *pk*, *fmi*, in the wing imaginal discs, suggesting that Rbf1 may directly control these genes. Analysis of the effect of overexpression of Rbf1 on the cloned promoter fragment of a core PCP gene, *vang/strabismus* in Kc cells indicate that *vang* is a physical and a direct target of the retinoblastoma protein. Our study suggests that Rbf plays a critical role in controlling cell polarity. Given the spectrum of mutations associated with the Rb family proteins in most cancer cell types, we propose that RB family inactivation contributes to enhanced metastatic potential of cancer cells..

799C

Genome-wide effects of retinoblastoma family proteins on gene expression and chromatin state in *Drosophila melanogaster* development. Irina Pushel[1], Sandhya Payankaulam[1], Rima Mouawad[2], David Arnosti[1,2]. 1) Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI; 2) Cellular & Molecular Biology, Michigan State University, East Lansing, MI.

The retinoblastoma (Rb) family of proteins plays an essential role in regulating growth and development. Misexpression of these proteins leads to developmental defects and has also been implicated in human cancers. The *Drosophila* homologs Rbf1 and Rbf2 have been shown to cause developmental defects when wild-type and mutant variants have been overexpressed in select tissues. In particular, mutations in the instability element appear to induce apoptosis, while others seem to have a proliferative phenotype. Our results suggest that some Rb mutations may induce a neomorphic phenotype, contributing to our understanding of human cancers. The aim of this study is to develop an understanding of biochemical effects of Rbf1 mutants through analyzing the effects of overexpression on gene regulation and development. Using a set of previously characterized Rbf1 mutants driven by a heat-shock promoter, the effects on chromatin of early expression of these mutants can be evaluated and compared to wild-type Rbf1 and Rbf2. By analyzing the effects on adult phenotype and ChIP-seq data, we can use information about mutant binding throughout the genome and chromatin state to elucidate the downstream effects of such mutations in disease and development.

800A

Identification of potential Stat92E target genes that affect hematopoietic output in *Drosophila*. Aditi Vyas, Soichi Tanda. Biological Sciences, Ohio University, Athens, OH.

A dominant mutation in the *Drosophila* Jak (or hop) gene called hop^{Tum} causes an increase in the Jak/Stat pathway activity level and results in a tenfold increase in the total hemocyte count. When we removed the negative regulator of the Jak/Stat pathway in the hop^{Tum} background, it further increased the pathway activity as supported by increased expression of the downstream target gene *Socs36E*, but surprisingly caused a reduction in the total hemocyte count. This suggests that the *Drosophila* Stat protein, Stat92E, controls expression of two sets of target genes; the low threshold genes (LTG), expressed at moderate levels of the pathway and the high threshold genes (HTG), activated at much higher levels of pathway activity. Our hypothesis is that the LTGs stimulate hemocyte proliferation, while the HTGs limit it. Our hypothesis predicts that at moderate levels of the pathway activity, expression from the HTGs is negatively regulated by transcriptional repressors. We thus screened LOF mutants of known co-repressors and repressors in the hop^{Tum} background and found that loss of *CtBP* as well as *Su(H)*, drastically decreased the hemocyte count. These results lend support to our hypothesis. Then, using *in silico* approaches, we aimed to search for possible regions in the *Drosophila melanogaster* genome that have Stat92E as well as repressor binding sites. A scan for presence of Stat92E and repressor sites in the CtBP ChIP-chip modENCODE dataset was performed using the bioinformatics tool Target Explorer. We also performed another genome wide scan, for Stat92E and Su(H), using the publicly available FIMO tool. Both these scans resulted in a final list of 30 potential Stat92E target genes that could have effects on hematopoiesis. RNAi against these candidates was performed in thehop^{Tum} background. Genes whose RNAi caused reduction in hemocyte count were selected as LTGs and those that caused a higher hemocyte count were selected as HTGs. We tested the expression of selected genes using qPCR assay in 8 different genetic backgrounds. Genes such as *Socs36E, lama* and *IntS14* that had a significantly higher expression in the hop^{Tum}; *CtBP* mutant background than thehop^{Tum} background have been selected as potential HTGs.

801B

***Groucho* and *Rpd3-Caf1* regulate *EGFR* signaling by repressing *rhomboid* expression during *Drosophila* development. T. Zhang**, W. Du. University of Chicago, Chicago, IL.

During the development of *Drosophila* imaginal discs, EGFR signaling is dynamically regulated and high level of *EGFR* activities are only exist in cells that are undergoing differentiation such as the photoreceptors in eye discs, sensory organs in antennal or leg discs,

and wing veins and margins in wing discs. The dynamic patterns of high level of *EGFR* activities in these cells are determined by the expression of *Rhomboid* (*Rho*), a membrane serine protease required for *EGFR* ligand processing. Previous studies indicated that transcriptional factors related to neural development such as *Atonal* (*Ato*) and *Daughterless* (*Da*) regulated *rho* expression in eye discs. Very little is known about how the expression of *rho* is regulated and whether there are common regulatory mechanisms regulating *rho*expression in different tissues. In a *Drosophila* genetic screen, we found that loss of *Groucho* (*Gro*), an evolutionally conserved transcriptional corepressor, induced ectopic expression of *rho* in the morphogenetic furrow of developing eye discs. Epistasis analysis showed that transcription factor *Da* but not *Ato* was necessary for *rho* expression in *Gro* deficient cells. Interestingly, while knockdown of the histone deacetylase *Rpd3* alone did not affect *rho* expression, double knockdown of *gro* and *Rpd3* synergistically induced ectopic *rho* expression in anterior eye discs as well as in antennal, wing, and leg discs. Furthermore, Knockdown of *gro* together with subunits of *Caf1,* an epigenetic complex that binds *Rpd3*, also induced synergistic *rho* expression while double knockdown of *Rpd3* and subunits of *Caf1* did not. Our studies reveal that transcriptional repression by *Gro* and *Rpd3-Caf1* is a general regulatory mechanism for *rho* expression and EGFR signaling in diverse cell types in developing imaginal discs.

802C

A novel 899bp enhancer regulates Vestigial expression in the myoblast population of the notal region of the wing disc. Elizabeth Clarke, Tyanna Lovato, Richard Cripps. Biology, University of New Mexico, Albuquerque, NM.

In adult Drosophila, the direct flight muscles (DFMs) and indirect flight muscles (IFMs) arise from the myoblast population of the notal region of the wing imaginal disc. Within the myoblast population, there are gene expression differences between the progenitor cells for the IFMs and DFMs, but there is still much to learn about the factors that specify each myoblast type. One difference between DFM and IFM populations is expression of Vestigial (Vg), a nuclear protein, which shows higher expression in IFM progenitors. Vg has mammalian orthologs that promote skeletal muscle differentiation and yet surprisingly little attention has focused on the regulators and targets of Vg in any system. Vg expression in the myoblasts is regulated through a novel 899 bp enhancer region specific to the wing disc myoblasts. Vg expression in the myoblasts is dependent on Twist (Twi). We have characterized Twist regulation of the Vg enhancer. We will also used this enhancer as a driver of DsRed for FACS isolation of the IFM progenitor cells for RNAseq analysis.

803A

Identification of an evolutionarily-conserved CRM that recapitulates Scr expression in the primordia of the T-row and sex comb bristles of T1 leg. Ece Eksi[1], Christopher McCallough[1], Artyom Kopp[2], Olga Barmina[2], Teresa Orenic[1]. 1) Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Department of Evolution and Ecology, University of California, Davis.

Our lab has previously shown that upregulated expression of the Hox gene, Sex combs reduced (Scr), marks the T-row primordia and sex comb bristles in the first pair (T1) of Drosophila melanogaster legs (Joshi et al., 2006, Shroff et al., 2007). We have identified a cis-regulatory module (Scr-E-CRM) that recapitulates Scr expression in the primordia of the T-row and sex comb bristles of T1 legs. We have initiated an analysis of putative transcription factor binding sites that might be responsible for activating transcription directed by the Scr-E-CRM. In this work, we analyze the contribution of 3 highly-conserved putative Distalless (Dll) binding sites in activating reporter expression from the Scr-E-CRM and show that the integrity of two of those sites are indispensable for recapitulation Scr expression pattern in T1 legs. Our results indicate that two Dll sites in the Scr-E-CRM are separately responsible for the full spatio-temporal expression pattern of Scr expression and for increasing the signal intensity from the reporter expression.

804B

***REDfly*: The Regulatory Element Database for *Drosophila*. Marc S. Halfon**[1,2,3,4,5], Steven M. Gallo[4,6]. 1) Department of Biochemistry, University at Buffalo, Buffalo, NY; 2) Department of Biological Sciences, University at Buffalo, Buffalo, NY; 3) Department of Biomedical Informatics, University at Buffalo, Buffalo, NY; 4) New York Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY; 5) Department of Molecular and Cellular Biology and Program in Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 6) Center for Computational Research, University at Buffalo, Buffalo, NY.

The REDfly database is a highly-curated portal for *Drosophila cis*-regulatory data containing records for empirically validated *cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs) curated from the published literature. REDfly includes all sequences reported as functionally tested in a transgenic reporter gene assay regardless of whether they showed regulatory activity or have activity redundant with other, shorter regulatory sequences. Graphical views show the position of each CRM within its genomic locus, and the location of each CRM with respect to its associated gene is provided. Curation of TFBSs includes sites identified by electrophoretic mobility shift assay (EMSA, "gel shift"), DNAase I footprinting, and high-throughput yeast one-hybrid assays. REDfly currently covers more than 670 publications and contains more than 11,600 records of reporter constructs regulating over 500 genes, including over 5500 "minimal" CRMs, and over 2000 TFBSs. Extensive abilities exist for database searching and results filtering. Forthcoming enhancements over the next year include complete migration to *Drosophila* release 6 sequence coordinates, significantly improved search and download capabilities, addition of ChIP-dervied TFBSs, and curation of an anticipated several thousand additional CRMs. REDfly provides a comprehensive source of *Drosophila cis*-regulatory data and is a powerful platform to facilitate high-throughput experimental and computational studies of gene regulation. REDfly is freely accessible at http://redfly.ccr.buffalo.edu. .

805C

Do regulatory interactions that result in repression of transcription elongation dominantly interfere with activation by other enhancers at the same promoter? Michael Higgins[1], Saiyu Hang[2], Haiyue Zhang[3], John Peter Gergen[4]. 1) Graduate Program in

Biochemistry and Structural Biology, Stony Brook University, Stony Brook, NY; 2) Postdoctoral Fellow, Boston Children's Hospital, Boston, MA; 3) Nanjing University, Nanjing, China; 4) Department of Biochemistry and Cell Biology and the Center for Developmental Genetics, Stony Brook University, Stony Brook, NY.

The *sloppy paired* gene presents a good model for studying regulation of gene expression by Runt. Two distinct cis-regulatory modules (CRMs) upstream of the *sloppy-paired-1* gene (*slp1*), the distal (DESE) and proximal (PESE) early stripe elements recapitulate the endogenous *slp1* expression pattern in a *lacZ* reporter construct in a manner not expected from the addition of their respective patterns. Experiments that investigate the mechanism of DESE and PESE regulation by the pair-rule transcription factors lead to a proposal that repression of a CRM that involves a block to transcription elongation prevents other CRMs from activating transcription at the same promoter. We have generated composite reporter gene constructs containing CRMs involved in patterning on both the A-P and D-V axis to test this hypothesis. Expression of these constructs both in wild-type embryos, as well as in response to ectopic expression of factors that block the release of a paused Pol II into an elongating complex provides further evidence that this mode of repression dominantly interferes with the ability of other CRMs to drive expression at this same promoter.

806A

Expression of *eyes absent* in the developing retina is controlled by a single enhancer composed of 3 separate *cis-regulatory* elements. Bonnie M. Weasner, Brandon P. Weasner, **Justin P. Kumar**. Dept Biol, Indiana Univ, Bloomington, IN.

The specification of the Drosophila compound eye is dependent upon the *eyes absent* (*eya*) locus as the loss of *eya* leads to a complete block in eye specification. Previous studies of the viable *eya*^{2}mutant allele concluded that a single 322bp genomic enhancer controls the complete spatial and temporal expression of *eya* in the retina. We uncovered 4 additional *cis-regulatory* elements within the *eya*locus that are capable of driving expression of a lacZ reporter in patterns similar, but not identical, to that of endogenous *eya*. Two of these elements flank the extant 322bp enhancer while the others are located within the first intron and 3' UTR, respectively. A reporter consisting of the extant enhancer and the two flanking elements ("upstream enhancer") mimics complete endogenous *eya* expression exactly, suggesting these elements work cooperatively to produce robust *eya* expression. Rescue experiments show that the upstream enhancer is capable of fully rescuing *eya*^{2} while the other elements, including the extant enhancer, support only a partial rescue of the no-eye phenotype. We have found that one of the new upstream elements is responsive to the misexpression of the known RD network genes, *teashirt* (*tsh)*, *tiptop* (*tio)* and *homothorax (hth)*. Tsh and Tio can activate the element while Hth represses its activity suggesting that these genes regulate *eya* expression in the developing retina through the upstream enhancer. Finally, we have determined that the no-eye phenotype of *eya*^{2} is due to a disruption of the upstream enhancer. If the two enhancers flanking the extant enhancer are fused together to simulate the genomic architecture of the *eya*^{2} mutant, then these enhancers can no longer recapitulate *eya* expression. When the spacing between these enhancers is restored by placing 322bp of exogenous DNA, reporter expression is restored. This fragment does not show activation of a reporter on its own suggesting that the spacing of the upstream elements is critical to function.

807B

***Patched* together: *cis*-regulatory control of the Hedgehog response. David Lorberbaum**[1,2], Andrea Ramos[2], Scott Barolo[1,2]. 1) Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI; 2) Cell and Developmental Biology, University of Michigan, Ann Arbor, MI.

A small number of cell signaling pathways are responsible for generating the cellular diversity required for all organisms to survive. One of these pathways is known as the Hedgehog (Hh) signaling pathway and is essential for patterning multiple tissues during development and is equally important for maintaining homeostasis in adult tissues. Regardless of context, the immediate output of Hh signaling is the activation or repression of target genes, all of which are directly regulated by Cubitus interruptus (Ci). This transcription factor functions through binding to enhancers, or *cis*-regulatory regions of DNA, to regulate levels of target gene transcription. Of the known Hh target genes, one of the best studied is *patched* (*ptc*), which encodes the Hh pathway receptor and is activated in all Hh-responding cells. Previous work has identified a promoter-proximal*ptc* enhancer and demonstrated that it requires three highly conserved consensus Ci binding sites to activate *ptc* transcription in the wing, a context that requires Hh for proper patterning. Despite its ability to respond in the wing, this enhancer is not sufficient to respond to Hh signaling in all relevant contexts. To understand how Hh target genes, like *ptc*, are transcriptionally regulated in all tissues, we have characterized several temporally and spatially regulated Hh responsive enhancers using stably integrated transgenic GFP reporters in *Drosophila melanogaster*. Most of these enhancers do not require consensus Ci binding sites, but do rely on non-perfect, low affinity sites to activate transcription. These data suggest that the transcriptional regulation of *ptc* is highly context dependent, requires Ci input from several different enhancers, and provides new information that better defines how Hh target genes are regulated in different developmental contexts. .

808C

A comparison of transvection effects at the *Men* and *Tpi* loci in *Drosophila melanogaster*. Patrick D. O'Donnell, Thomas J. S. Merritt. Laurentian University, Sudbury, Canada.

The conventional, *cis*-only, model of enhancer-promoter interactions is being challenged as more and more examples of interactions in *trans* between homologous chromosomes are being described. One specific class of *trans*-interaction is the pairing dependent mechanism "transvection", in which the regulatory elements from a promoter-less allele act on an enhancer-less homolog to up- or down-regulate expression of a locus. We have described both cis- and trans-regulation at the *Malic enzyme* (*Men*), demonstrating the sensitivity of transvection to environment and genetic background. In an effort to expand and clarify our model of transvection, we have

been exploring a second system at the *Triose-phosphate isomerase* (*Tpi*) locus. While the mechanism of transvection at the two loci is similar in many ways, the distinct regulatory regions in *Men* and *Tpi* are allowing us to create a more general model for the basic mechanism of transvection by comparing and contrasting the details of how each locus is regulated in *trans*.

809A

Control of transcription dynamics by shadow enhancers of Kruppel. Clarissa Scholes[1,2], Alvaro Sanchez[3], Angela DePace[1]. 1) Graduate Program in Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138; 2) Department of Systems Biology, Harvard Medical School, Boston, MA 02115; 3) Rowland Institute at Harvard, Cambridge, MA 02142.

In animals, gene expression is often controlled by multiple enhancers, each of which directs a portion of the overall pattern in space and time. In some cases different enhancers drive overlapping patterns, which suggests that they can impinge on the same promoter in the same cells at the same time; these are known as shadow enhancers. How a promoter interacts with multiple enhancers is unclear. We have addressed this question using shadow enhancers controlling expression of Kruppel, a transcription factor important for anterior-posterior axis patterning in *Drosophila*. We used the MS2 system to measure transcription dynamics in live embryos to determine how the shadow enhancers of Kruppel interact with the promoter and with one another to govern its expression in the early embryo. We contextualize our results using a kinetic modeling framework to explore the range of computations that can be achieved by two enhancers acting on the same or different steps in transcription initiation. We are extending this analysis to orthologous enhancers of Kruppel to understand how the allocation of activity between shadow enhancers has changed over evolutionary time.

810B

Genome wide identification of cis-regulatory elements from small cell population: Insights from the drosophila cardiac tube. Denis Seyres[1], Yad Ghavi-Helm[2], Celine Guichard[3], Magali Torres[1], Charles Girardot[2], Eileen Furlong[2], Laurent Perrin[1]. 1) TAGC U1090, Marseille, cedex 9, France; 2) European Molecular Biology Laboratory (EMBL), MeyerhofstraBe 1, 69117 Heidelberg, Germany; 3) Child and hess institute [Mount Sinai], 1468 madisson avenue, New York, USA.

Decrypting tissue specific gene expression regulation and cis-regulatory circuits is a central issue to understand organogenesis. To get an holistic view of the regulatory landscape at play requires the acquisition of genome-wide informations. However generating tissue-specific genomic data from scarce cell populations in the developing embryo remains a big challenge.
To address this we focused on the developing cardiac tube, being made up of only 104 myocytes representing less than 0,5% of the whole embryo, while focusing more precisely on cardioblasts diversification and differentiation, the latest steps of heart organogenesis. Chromatin modifications are associated with different aspects of gene expression including enhancers and promoters activity states. Using a highly specific cardiac reporter, we adapted a recently described method for cell type specific analysis of chromatin states (BITS-ChIP-seq; Bonn et al, 2012) to very rare cell populations. We identified cardioblasts-specific active enhancers and promoters by analysing two histone modifications (H3K27ac and H3K4me3) at a genome wide scale. In addition, to analyse heart specific gene expression levels we also generated RNA-seq data from FACS sorted cardioblasts. In silico and in vivo validations confirmed the accuracy and specificity of our data. Finally we are using machine learning approaches with these genomic data to detect the main features of cardiac specific enhancers with the aim of getting new insights regarding the gene regulatory network governing cardioblasts differentiation.

811C

Enhancers by design. Ben Vincent[1], Meghan Bragdon[1], Garth Ilsley[2], Zeba Wunderlich[1], Javier Estrada[1], Angela DePace[1]. 1) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) Okinawa Institute of Science and Technology Graduate University, Japan.

A grand challenge in both molecular biology and medicine is to engineer regulatory sequences that drive gene expression in any cell type of interest. While many scientists rely on existing regulatory sequences from animal genomes, we would like to design sequences that drive novel expression patterns. This goal requires a theoretical framework to predict how to encode new patterns given the constraints of a regulatory network. To this end, we have developed a computational approach that predicts a new way to encode the seven stripe *even-skipped* (*eve*) expression pattern in the *Drosophila melanogaster* embryo by engineering existing *eve* enhancers. We validated these predictions by encoding two novel patterns. To remove part of an existing pattern, we added repressor binding sites to the*eve4+6* enhancer and created a sequence that generated stripe 6 alone. To combine patterns, we added activator binding sites and removed repressor binding sites within the *eve5* enhancer and generated stripes 2 and 5 together. Our results demonstrate that complex developmental enhancers can be rationally engineered to generate precise, novel patterns, and we are currently using genome engineering to determine the fitness consequences of rearraginging the regulation information in the *eve* locus.

812A

A massively parallel reverse genetic screen for early embryonic patterning mutants. Peter Combs[1], Michael Eisen[2,3]. 1) Biophysics Grad Group, UC Berkeley, Berkeley, CA; 2) Department of Molecular and Cell Biology, UC Berkeley, CA; 3) Howard Hughes Medical Institute, UC Berkeley, CA.

Genome wide sequencing of entire genomes has become commonplace, but the understanding of how those genomes ultimately specify cell fate during development is still elusive. While a handful of developmentally important *Drosophila* genes have had their regulation deeply investigated, extending insights from those loci genome wide is still a major challenge. The developing embryo provides a unique opportunity to study the role of gene expression in pattern specification; the precise and consistent spatial positioning of key transcription factors essentially provides separate transcriptional-readout experiments at a critical point in

development. We used cryosectioning of single *Drosophila melanogaster* embryos at the blastoderm stage combined with RNA-seq to screen for spatially-varying regulation of transcription. Whereas previously we have only screened wild type embryos, here we present data from dosage mutants for key maternally provided regulators, including depletion of *zelda* and *hunchback* and both overexpression and depletion of*bicoid*. We have found a number of stereotyped patterning changes, even to these TFs with different localization patterns. Furthermore, the *bicoid* dosage series of 0x, 1x, and 2.6x protein levels allows us to probe regulatory input functions of every bicoid responsive gene. Overall, our results fill in key gaps in knowledge that non-spatially resolved approaches cannot address. We have probed the regulatory impact of known master-regulators across all of their targets, rather than just a handful of well characterized sites.

813B

A transcriptomic approach to sex determination and the maternal-to-zygotic transition in *Nasonia*. Deanna Arsala, Jeremy Lynch. Biological Sciences, University of Illinois at Chicago, Chicago, IL.

During early insect development, the embryonic blastoderm is a crucial stage in which tissue fates are specified along the major axes as the embryo transitions from maternal to zygotic control of development. Concurrent to this process, the sex of the developing embryo is established through cell autonomous mechanisms that are dependent on the regulation and activation of the zygotic genome. *Nasonia* is a genus of small parasitoid wasps. Sex determination in these wasps is particularly interesting, as they are haplodiploid, meaning that fertilized eggs will yield diploid females, whereas unfertilized eggs will yield haploid males. However, it has been clearly demonstrated that sex identity is not directly dependent upon the ploidy of the embryo but rather on levels of the female specific splice form of *Nv-transformer*, a sex-specific splicing factor in the sex-determination cascade of *Nasonia*. Since direct genomic imprinting of maternal *Nv-tra* has been ruled out, recent studies have suggested an unknown factor (termed *womanizer (wom)*) may be responsible for the zygotic activation of *Nv-tra*, during the maternal to zygotic transition. We are further characterizing the *Nasonia* sex determination network by functionally testing differentially expressed transcripts to uncover the roles of genes that are sexually regulated in the blastoderm stage. Additionally, we are also interested in how the maternal-to-zygotic transition (MZT) operates in haplodiploid organisms to establish sex identity and maintain proper development. We will characterize the *Nasonia* MZT using RNA-seq on hybrid *Nasonia* embryos and functionally analyzing homologs of the two major players that have been identified in the MZT of *Drosophila, Smaug* and *Zelda*. .

814C

Probing Structure/Function Relationships in the *gurken* 5′ UTR. Phillip A. Frankino[1], Jacob A. Merle[1], Ramses Rodriguez[1], David DiPalma[1], Allison H.H. Martin[1], Matthew A. Fountain[2], Scott B. Ferguson[1]. 1) Biology Department, Fredonia, NY; 2) Biochemistry and Chemistry Department, Fredonia, NY.

The protein Gurken, *grk*, is a developmentally important morphogen that helps to determine dorsal/ventral polarity in the developing oocyte of *Drosophila melanogaster*. Like many mRNAs, *grk* is translated by the cannonical binding of the pre-initiation complex to the 5′ 7-methyl-guanasine cap. We hypothesize that *grk* mRNA also has structural features in the 5' UTR that facilitate internal ribosomal entry site (IRES) activity. SHAPE analysis suggests that there are two hairpin stem loops in the 5′ UTR that coincide with the general characteristics of known IRES structures. *In vivo* reporter constructs with mutations in the stem loops have been generated and transgenically inserted in Drosophila. We will present data that will establish the role of these structures in the 5′ UTR and the effect that they have on the level of *grk* translation.

815A

Regulation of expression of RNP-4F splicing assembly factor in *Drosophila melanogaster*. Sushmita Ghosh, Lindsey Abraham, Jack Vaughn. Biology, Program in Cell Molecular and Structural Biology, Miami University, Oxford, OH.

The *Drosophila rnp-4f* gene encodes RNP-4F protein which is a splicing assembly factor. The *Drosophila rnp-4f* gene codes for two mRNA isoforms (“long” and “short”) which differ by a 177-nt sequence (caused by alternative splicing in the 5′-UTR within intron 0 and exon 2) that forms an evolutionarily conserved stem-loop. Several observations suggest that *rnp-4f* expression may be regulated by a feedback pathway. Northerns and RT-PCR studies suggest that there is a developmental switch that controls the levels of the two isoforms. Northerns show that the long mRNA isoform level peaks in the mid-embryo stage. Westerns and RT-PCR show that high levels of RNP-4F protein correspond to elevated levels of the short *rnp-4f* mRNA isoform during very early embryo stages and late stages of fly development. Evolutionary conservation of the *rnp-4f* stem-loop and developmental regulation of alternative transcript levels suggest functional significance of this 5′-UTR stem-loop structure. RNA electrophoretic mobility shift assay using *in vitro* transcribed RNA (*rnp-4f* 5′-UTR 177-nt stem-loop) and whole embryo protein extract from wild-type embryos and *dADAR* mutant embryo protein combined with qPCR analysis and RNAi studies suggest that dADAR is one of the two proteins that bind to the stem-loop. However, the identity of the other protein remains unknown. A structural study has revealed that there exists a conserved sequence on U6-snRNA to which the RNP-4F chaperone may bind. A stretch of 12 nucleotides within the 5′-UTR 177-nt in the *rnp-4f* mRNA shares significant sequence similarity with the conserved binding site on U6-snRNA. Another level of similarity is that in both cases the consensus sequence lies within a long stem-loop secondary structure. Based on all our findings we hypothesize that RNP-4F binds to the stem-loop in the *rnp-4f* mRNA 5′-UTR (long isoform) and regulates its own expression *via* a feedback pathway. A UAS-Gal4 gene expression system is described which is being used to test this hypothesis. We are currently studying the role of RNP-4F in autoregulating its own expression.

816B

Measuring the impact of microRNAs on *in vivo* gene expression at single cell resolution: Can microRNAs suppress noise in gene expression? R. Giri, R. Carthew. Department of Molecular Biosciences, Northwestern University, Evanston, IL.

MicroRNAs are repressors of gene expression that prevail throughout the animal kingdom. An emerging theory suggests that microRNAs might suppress variation in expression of target proteins between cells of a tissue. An elegant context to test this theory is the sensory organ precursor (SOP) cell fate switch in *Drosophila.* SOP formation is highly invariant between individuals due to precise control of expression of a pro-SOP transcription factor, Senseless (*sens*). *Sens* translation is directly repressed by microRNA miR-9a. Loss of the *mir-9a* gene increases variation in the number of cells that switch to an SOP fate - perhaps due to increased heterogeneity of Sens protein levels in cells of the developing imaginal discs. To test this hypothesis, BAC recombineering was used to create genomic constructs of*sens* fused in frame to sfGFP or mCherry, and these completely replaced the endogenous *sens* gene (deleted by null mutations). We perturbed miR-9a regulation of the transgenes by combinatorially mutating the two miR-9a binding sites in the 3'UTR. We measured fluorescence levels in thousands of individual wing disc cells using quantitative microscopy and automated image capture. Analysis indicates that, as expected, average *sens* protein expression modestly increased with loss of miR-9a sites. However, rather than a simple uniform shift in the distribution of protein levels across all cells, there is a specific increase in Sens protein levels in cells that otherwise would have expressed a stable intermediate level of Sens. Thus, greater variation in Sens protein expression is observed in wing disc cells. To precisely correlate microRNA repression with intrinsic gene expression noise, we plan to employ allele-specific two-color expression measurements using genomic *sens* alleles with different numbers of intact mir-9a sites. This will enable us to measure *in vivo* gene expression variation as well as to directly test a novel regulatory function that this microRNA might occupy.

817C

Identifying Novel Drosophila Mutations That Affect *gurken* Translation in *spindle B* Mutants. John Hasper, Breanna Meyers, Malachi Blundon, Austie Lawrence, Scott Ferguson. Biology, SUNY Fredonia, Fredonia, NY.

We have taken advantage of positional cloning and next-generation sequencing approaches to identify candidate mutations in various independent lines from a forward genetic screen for regulators of dorsal ventral patterning during Drosophila oogenesis. Oogenesis is dependent on precise translational control and localization of numerous morphogens within the oocyte to achieve faithful patterning. Gurken, (Grk) is one such protein and is responsible for specification of the dorsal/ ventral axis. Mutations in the *spindle-B gene* results in inefficient *gurken* translation due to activation of a meiotic DNA damage checkpoint that inhibits the Vasa RNA helicase, an essential *grk* translation factor. Thirty nine unique mutants were identified from an EMS mutagenesis of the third chromosome in a *spn-BBU* mutant background. Lines were screened for their ability to suppress the ventralized *spn-BBU* phenotype and therefore stimulate *grk* translation by novel signaling pathways. Eggs laid by homozygotes from each of the isogenized lines were scored for their dorsal/ventral polarity and compared to those of the control group of *spn-BBU* homozygotes. The best suppressor lines were subject to whole-genome re-sequencing using the Illumina HiSeq 2000 platform. These data have allowed us to generate a list of candidates for the causative mutations in the suppressor lines. To complement the data garnered from the Illumina sequencing, a positional cloning experiment was performed with the use of SNP markers. The positional cloning experiment has enabled us further refine the list of candidates. We are currently evaluating this shorter list of candidate mutations to elucidate the mechanism by which *grk* translation has been restored.

818A

A heterodimer-based regulatory role for *Drosophila* truncated dADAR protein isoform function. Fatemeh Kohram, Sushmita Ghosh, Jack Vaughn. Biology, Program in Cell Molecular and Structural Biology, Miami University, Oxford, OH.

The single-copy *Drosophila ADAR (dADAR)* gene encodes a dsRNA-dependent adenosine deaminase. In embryos, two major mRNA isoform classes exist, which are full-length (FL) and truncated (TR). FL contains the deaminase catalytic domain and TR does not. It has been shown that *in vitro* synthesized FL isoform is catalytically active, but is nearly completely inactive *in vivo*. The mechanism(s) for inactivity of FL during embryo development are incompletely known, and nothing is known about the function of the TR isoform. It has been shown by others that full dADAR activity requires homodimers in *Drosophila*, and that heterodimers between FL and inactivated FL isoforms have greatly reduced activity. It is also known that in *C. elegans* heterodimers between catalytic and non-catalytic isoforms regulate ADAR activity. Here, we present evidences in support of a model for a heterodimer-based regulatory role in *Drosophila* TR isoform function. Importantly, the N-terminal region required for dimerization in *Drosophila* dADAR is present in both the FL and TR isoform, as are the two dsRNA-binding domains. DIG *in situ* hybridization shows that FL and TR mRNAs co-localize in the developing embryo, notably within the brain and ventral nerve cord. 3'-RACE shows that both mRNA isoforms are present across all embryo developmental stages, but that the TR isoform is absent in adults where editing activity is highest. RT-PCR studies show that the ratio in abundance of TR to FL mRNA is 0.35/0.65 in pooled embryo stages. Westerns show that encoded dADAR protein for both isoforms is present during all embryo stages, and that their relative abundance remains constant. Westerns show that the TR protein isoform is present in embryos from all fly species tested, at least as far back as *D. americana* with a 40×10^6 year divergence time, suggesting a conserved function for this isoform. Taken together, these results support a model in which heterodimers between the two major isoform classes negatively regulate dADAR catalytic activity during embryo development in *Drosophila*. Our current research centers on testing this model.

819B

The Wright stuff: reinventing path analysis reveals novel components of the sex determination hierarchy in *D. melanogaster*. Justin Fear[1,2], Michelle Arbeitman[3], Matthew Salomon[4], Justin Dalton[3], John Tower[4], Sergey Nuzhdin[4], Lauren McIntyre[2]. 1) Genetics & Genomics, Univ Florida, Gainesville, FL; 2) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 3) Department of Biomedical Sciences, Florida State University, Tallahassee; 4) Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

The *Drosophila* sex determination hierarchy consists of a pre-mRNA splicing cascade that produces sex-specific transcription factors directing somatic sexual dimorphism. While much is known about sex hierarchy it is still incomplete. We pioneer an approach using natural genetic variation to expand our knowledge of the sex hierarchy and provide a generalizable method to expand gene regulatory networks (GRNs), particularly in human populations. Two studies from *Drosophila* female head tissue – the DSPR collection (alleles from 15 natural variants) and F1-hybrid data (alleles from heterozygotes of 75 isogenic lines and 1 laboratory strain) – are used in a structural equation model analysis. Here we expand the sex hierarchy GRN by adding a novel links between genes in the pathway and by adding genes to the pathway. A link from *fruitless* to *Sex-lethal* is found in both populations, which is supported by the presence of *fru* binding sites in the *Sex-lethal* locus. The splicing factors *msl-2*, *B52*, and *Rm62*are identified by the models as downstream targets of *Sxl*. Of the 1390 genes placed in the expanded GRN, there is enrichment for genes with sex-biased splicing. As with other population-genetic analyses, the number of alleles limits the number of observable interactions. Network expansion was clear in the F1 hybrid population, with an average of twice the number of alleles as the DSPR. Independent studies in adult female head tissues using *doublesex* and *transformer* mutant strains supports many novel connections, including evidence for a link between the sex hierarchy and metabolism, with the inclusion of*InR* in the sex hierarchy GRN.

820C

Improved statistical methods enable greater sensitivity in rhythm detection for genome-wide data. Alan L Hutchison[1,2,3], Mark Maienschein-Cline[4], Andrew H. Chiang[3], S. M. Ali Tabei[3], Herman Gudjonson[2,3], Neil Bahroos[4], Ravi Allada[5], Aaron R. Dinner[2,3,6]. 1) Medical Scientist Training Program, University of Chicago, Chicago, IL; 2) Graduate Program in Biophysical Sciences, University of Chicago, Chicago, IL; 3) James Franck Institute, University of Chicago, Chicago, IL; 4) Center for Research Informatics, University of Illinois at Chicago, Chicago, IL; 5) Department of Neurobiology, Northwestern University, Evanston, IL; 6) Department of Chemistry, University of Chicago, Chicago, IL.

Robust methods for identifying patterns of expression in genome-wide data are important for generating hypotheses regarding gene function. To this end, several methods have been developed for detecting periodic patterns. We improve one such method, JTK_CYCLE, by explicitly calculating the null distribution such that it accounts precisely for multiple hypothesis testing and, in turn, by including non-sinusodial reference waveforms. We term this method empirical JTK_CYCLE with asymmetry search, and we compare its performance to JTK_CYCLE with Bonferroni and Benjamini-Hochberg corrections for multiple hypothesis testing, as well as to five other rhythm detection methods: cyclohedron test, address reduction, stable persistence, ANOVA, and F24. We find that ANOVA, F24, and JTK_CYCLE consistently outperform the other three methods when data are limited and noisy; empirical JTK_CYCLE with asymmetry search gives the greatest sensitivity while controlling for the false discovery rate. Our analysis also provides insight into experimental design, and we find that, for a fixed number of samples, better sensitivity and specificity are achieved with higher numbers of replicates than with higher sampling density. Application of the method to detecting circadian rhythms in a metadataset of microarrays that quantify time-dependent gene expression in whole heads of *Drosophila melanogaster* reveals annotations that are enriched among genes with highly asymmetric waveforms. These include a wide range of oxidation reduction and metabolic genes, as well as genes with transcripts that have multiple splice forms.

821A

Using structural equation modeling to detect regulatory interactions in the Inr/Tor pathway. Felicia New[1], Michelle Arbeitman[2], Nicole Newell[2], Justin Fear[1], Lauren McIntyre[1]. 1) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) Department of Biomedical Sciences, Florida State University, Tallahassee, FL.

The insulin receptor (Inr) and target of rapamycin (TOR) pathway is a conserved signaling cascade in animals. It functions as a regulator of metabolism and growth (Grewal, 2009). The pathway regulates transcription through the regulation of FOXO, a downstream transcription factor. Genes within the core InR/Tor pathway show correlated gene expression patterns (Nuzhdin et al., 2009), however we know that mRNA level is only moderately correlated with protein concentration (Ideker er al., 2001). First, we are interested to see which regulatory interactions (e.g. transcriptional regulation, protein-protein complex, kinase signal propagation) are identified by SEMs. We expect to see strong relationships in the downstream pathway (i.e. transcriptional regulation via FOXO) and weaker relationships upstream (i.e. kinase signal propagation). Next, to investigate if there are changes to the regulatory network structure after mating, we will compare the SEMs of the networks of mated and virgin flies.

822B

An imaging-based approach to quantitative in vivo analysis of gene regulatory networks. Radoslaw Ejsmont, Bassem Hassan. VIB Center for the Biology of Disease, VIB, Leuven, Belgium.

Specification of cell identity is a multi-step process that is governed by key transcription factors, many of which have been identified, however their targets remain largely unknown or were only analyzed bioinformatically. It is also not clear yet, whether the binding of a transcription factor immediately results in expression of the target genes, thus ChIP approaches may result in false positives. In this

project we aim to identify the sequence of quantitative changes in gene expression which govern the development of the neural retina in *Drosophila* using a novel, imaging-based approach. The sequence of events in retinal differentiation is governed by 3 master control transcription factors, namely *Eyeless*, *Atonal* and *Senseless,* controling the transition from eye disc intermediate cell to R8 photoreceptor neurons. Within the network formed by these three factors, we are currently focusing on transcriptional control of *atonal* over its targets. We are utilizing the power of recombineering to tag all computationaly predicted *Ato* target genes with a novel transcriptional reporter that allows us to follow and quantify expression of the regulated genes*in vivo*. We have used genome engineering to create labeled null and wild-type alleles of *ato* in a manner enabling creation of homozygous wild-type and mutant clones using a novel FRT-based approach. Finally, we are developing an image processing pipeline to extract quantitative information from live imaging of eye-antennal imaginal discs and a framework enabling analysis of this unpreceeded spatio-temporal resolution transcriptomics data.

823C

Metabolic gene linkages to long non-coding RNAs and allele-specific expression. Alison Gerken[1], Justin Fear[1,2], Sergey Nuzhdin[3], Lauren McIntyre[1]. 1) Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) Genetics and Genomics Graduate Program, University of Florida, Gainesville, FL; 3) Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

Non-coding regions of the genome, including UTRs and intronic regions, have been shown to impact regulation of genome expression, including regulation of cancers and neurodegenerative disorders in mammals. Influence of these genomic elements on metabolic regulation may also influence metabolic diseases and categorization of long non-coding RNAs (lncRNA) with metabolic genes may provide us with insight on transcriptional regulation of metabolism. Within a heterozygous population there is variation in the amount of lncRNA regulation. Associations between lncRNA and *cis*-regulation have been found in tumor growth and transcription inhibition, as well as associations with ncRNAs and metabolic diseases such as Prader-Willi syndrome. We hypothesize a link between lncRNA variation and variation in *cis*-regulatory polymorphisms associated with several metabolic genes. Polymorphisms in *cis*-regulatory regions can be identified from allelic imbalance, the expression of one allele over the other, within an individual. Female *Drosophila* show substantial variation in metabolism following mating. We will use a population of F1-hybrids derived by crossing 68 recently isolated lines to a common laboratory strain of *Drosophila melanogaster* to assess allele specific expression and the association of lncRNAs with genes involved in metabolism.

824A

***Caudal* drives *Themira putris even-skipped* stripe 2 expression in *Drosophila melanogaster* ?** Ah-Ram Kim[1], **Pengyao Jiang**[2], Michael Ludwig[2,3], John Reinitz[2,3,4,5]. 1) MIT Computational Biology Group, Massachusetts Institute of Technology, Cambridge, MA; 2) Ecology & Evolution, University of Chicago, Chicago, IL; 3) Institute for Genomics & Systems Biology, University of Chicago, Chicago, IL; 4) Department of Statistics, University of Chicago, IL; 5) Department of Molecular Genetics and Cell Biology, University of Chicago, IL.

Many studies have shown that functional conservation of *cis*-regulatory elements does not require sequence conservation. Specifically, there is rapid turnover of binding sites in enhancers regardless of conserved gene expression between distantly-related species. Previously-identified *even-skipped (eve)* stripe 2 enhancer in Sepsidae fly *Themira putris (T. put)* can drive *eve* stripe 2 expression in*Drosophila melanogaster (D. mel)*, with little sequence similarity to its *D.mel* correspondence. Noteworthily, *T. put* stripe 2 enhancer lost most of its Bicoid binding sites, which factor is the major activator in *D. mel* stripe 2 enhancer. Using a computational model of transcription, we predict that in *T. put* stripe 2 enhancer, Caudal serves as a major activator and coactivator for driving stripe 2 expression in*D.mel*. We sought to test this hypothesis by examining reporter gene expression driven by the two enhancers in *caudal* null background. Either *D. mel eve* stripe 2 enhancer or *T. put eve* stripe 2 enhancer is put into both maternal and zygotic *caudal* null background. As predicted, *T. put eve* stripe 2 enhancer does not drive expression in *caudal* null background, while *D. mel* stripe 2 enhancer does. We reasoned that there are multiple ways of utilizing different configurations of *cis*-regulatory logic with the same transcriptional machinery and *trans*-acting factors to generate similar transcription output.

825B

Dissecting *Miscadestral pigmentation* (*Mcp*) in the *Abdominal-B* (*Abd-B*) locus: establishment of a Recombination Mediated Cassette Exchange (RMCE) platform using the CRISPR/Cas9 method. Mario Metzler[1,2], Martin Müller[1], Markus Affolter[1]. 1) Biozentrum, Zellbiologie, Universität Basel, Basel, Switzerland; 2) Fellowships for Excellence, Biozentrum, Universität Basel, Basel, Switzerland.

Abd-B is the most posterior gene in the bithorax Hox complex (BX-C) and is responsible for the segmental identity of the most posterior five para-segments of *Drosophila melanogaster*. The expression of *Abd-B* has to be tightly regulated, as mis-expression of the gene leads to homeotic transformations. The main players in the process of transcriptional *Abd-B* regulation are two types of elements: initiation and boundary elements. Initiation elements receive inputs from gap and pair-rule genes in a para-segment dependent way. Thus transcription of the *Abd-B* gene is activated in a spatially and temporally defined way. Boundary elements are less understood, but seem to play a role in the topological arrangement and epigenetic regulation of the *Abd-B* region. Mechanistically this is due to the presence of binding sites for insulator proteins and for proteins associated with Polycomb Response Elements (PREs). *Mcp* is a ~1kb DNA element situated between the *abdominal-A* and *Abd-B* genes. It is genetically defined by a set of three small overlapping deletions. The dominant *Mcp* phenotype is best seen in males where the normally unpigmented fourth abdominal segment acquires pigmentation that is specific to more posterior segments A5 and A6. Molecular studies have shown that the phenotype is due to ectopic expression of *Abd-B* in A4. Further experiments tested the function of various *Mcp* fragments in transgenic assays and assessed that it contains a

PRE, can act as an enhancer blocker and can mediate long distance contacts between two remote *Mcp* fragments. Until recently a more detailed study of*Mcp* at its endogenous location would have been difficult to accomplish. With the adaptation of the ΦC31 recombination and the CRISPR/Cas9 systems for *Drosophila* genetics, manipulation of *Mcp* in the context of the bithorax complex has become a realistic goal. We will present our approach towards that aim.

826C

Evolutionary rates of redundant enhancers. Anne Sonnenschein, Ian Dworkin, David Arnosti. Michigan State University, East Lansing, MI.

Computational and genomic experiments have suggested that 1/10 to ½ of *Drosophila* genes in embryonic development in Drosophila may have 'shadow enhancers': cis-regulatory elements with some degree of functional redundancy. Genetic redundancy generally relaxes selection and influences the rate of evolution. This suggests that shadow enhancers might contribute to enhancer turnover and rapid cis-regulatory evolution in *Drosophila*. However, this hypothesis is difficult to test given the high degree of conservation in *Drosophila* non-coding sequences, and the lack of a complete list of how many enhancers are associated with each gene. We have created a pipeline for identifying enhancers and predicting their associated expression patterns. This has been used to compose a list of enhancers associated with genes involved in embryonic development, and predict their degree of regulatory redundancy. These predicted enhancers are compared with orthologous sequences in related species, to test the influence of shadow enhancers on evolutionary rates.

827A

Determining the role of 3D nuclear architecture in stochastic gene expression. Kayla C Viets, Robert J Johnston Jr. Department of Biology, The Johns Hopkins University, Baltimore, MD. Chromosomes are organized in a complex manner within the nucleus. One essential aspect of nuclear organization involves the interaction of genomic loci across long nuclear distances through targeting to specific nuclear regions, chromosome looping, and gene pairing. Insulators and Polycomb response elements (PREs), DNA elements that are bound by insulator proteins and the Polycomb Group Complex, can mediate these interactions. The long-distance interaction of genomic regions plays an important role in regulating stochastic gene expression in the *Drosophila* retina, where the *spineless* (*ss*) gene is expressed in a random on/off manner in a subset of R7 photoreceptor cells. Two mechanisms control random *ss* expression. First, each copy of *ss* within a nucleus makes a random, independent decision to be either on or off. When expression states disagree (one copy on and one off), a second mechanism of Interchromosomal Communication (InterCom) coordinates *ss* expression so that both copies agree and are either both on or both off. This mechanism appears to require physical interactions between *ss* loci, as copies of *ss* colocalize within photoreceptor nuclei. Interestingly, InterCom does not appear to be position-dependent; intact copies of the *ss* locus can cross-regulate endogenous *ss* expression from heterologous positions in the genome. Additionally, InterCom requires the presence of certain insulators and PREs within the *ss* locus: deletions of specific PREs and insulators limit the range across which *ss* copies can perform InterCom or completely abolish InterCom function. These results suggest a mechanism in which insulators and PREs facilitate the physical pairing and expression coordination of copies of *ss*. .

828B

Transcriptional regulation of the Insulin Receptor gene in *Drosophila melanogaster*. Y. Wei, R. Gokhale, W. Henry, D. Arnosti. Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI.

The insulin-signaling pathway is a conserved signaling cascade that plays a major role in regulating metabolism and growth in diverse metazoan species. The insulin receptor (IR) functions to direct insulin to specific target tissues, and to initiate the response to the hormone. Deregulation of the *IR* gene has been found in type-II diabetes and in a number of tumors. *Drosophila* has one insulin-like receptor (InR) that is 35% identical to human IR. It is essential for development and is required for the formation of the epidermis and nervous system during embryogenesis. Natural variation of the *InR* gene is also associated with regional size variation and stress tolerance. The *InR* gene has three TSS, and nearly 40 Kb intron regions that may serve as binding sites for transcription factors. Previous studies have shown that *Drosophila* forkhead protein FOXO (dFOXO) and ecdysone receptor (EcR) directly target the *InR* gene, and effectively regulate its gene transcription in response to nutrient and steroid hormone. Our research identified, for the first time, that retinoblastoma family members, Rbf1 and Rbf2, strongly associate with the promoter-proximal region of the *InR* gene, and Rbf1 functionally represses the *InR*promoter *in vitro*. To identify the *cis*-regulatory elements (CREs) for Rbf1, dFOXO, and EcR, and systematically analyze their roles in controlling the *InR* gene expression, we generated a luciferase reporter library by dissecting the *InR* gene regulatory regions into 1.5 Kb fragments and fusing to the basal promoter of the *InR* gene. By *in vitro* assays, we found that those reporters had distinct basal expression levels, and differential behaviors in response to dFOXO/EcR over-expression or ecdysone treatment. In addition, some fragments also drove tissue specific expression *in vivo*. These suggest that the CREs in the *InR* gene control both the spatial and temporal patterns of the gene expression. In cooperate with bioinformatics, we will deciphering the "*cis*-acting code" of the *InR* gene, and identify the roles of the CREs *in vivo*. Our study will offer molecular insights into the nutrient growth-maturation coordination in *Drosophila* development and the deregulation of insulin receptor in multiple human tumors.

829C

Mechanism of *repo* Regulation in Glial Cells of *Drosophila melanogaster*. Jamie L. Wood, Bradley W. Jones. Dept. of Biology, University of Mississippi, University, MS.

During development cells change from unspecified to specified states. Cells of the nervous system begin as unspecified precursors and proceed along one of two developmental paths to become either neurons or glia. We seek to understand more about the genes that control this process using *Drosophila melanogaster* as a model system. Previous work from our lab and others has established the role of the master regulatory transcription factor Gcm in directing neuronal precursor cells to assume a glial fate. Gcm acts on many target genes, one of which is *reversed polarity (repo)*. *repo* is necessary for proper glial cell differentiation; once activated, its expression is maintained throughout the life of the fly through currently unknown mechanisms. We propose that *repo* expression is maintained in an autoregulatory manner, whereby Repo protein acts as a transcription factor on its own regulatory DNA sequence. Three canonical Repo binding sites (RBSs) are located within the 4.3 kb *repo cis*-regulatory DNA (CRD); using both *in situ* and *in vivo* expression systems, we have evidence that suggests Repo protein interacts strongly with one of these sites to induce the expression of reporter genes. Mutagenesis of this site results in a significant decrease of reporter gene expression in both systems. Further research will explore the role of the remaining two RBSs in the *repo* CRD. .

830A

The *cis*-regulatory logic controlling *Defective proventriculus* (*dve*), a critical regulator of *rhodopsin* expression in the *Drosophila* eye. Jenny Yan[1*], Gregory Goldberg[2], Robert Johnston[1]. 1) Biology, Johns Hopkins University, Baltimore, MD; 2) Immunology, Virology and Microbiology, The Rockerfeller University, New York, NY.

Development of sensory systems requires regulatory networks that govern cell-specific receptor gene expression, allowing organisms to react to environmental cues. The *Drosophila* compound eye consists of a mosaic of unit eyes, each containing six outer photoreceptors (PRs) (R1-R6) and two inner PRs (R7 and R8). The outer PRs express motion-detecting Rhodopsin 1 (Rh1), while the inner PRs express color-sensitive Rh3-Rh6. The transcription factor (TF) Defective Proventriculus (Dve) is a critical regulator of Rh expression, acting as a repressor for Rh3, Rh5, and Rh6. Upstream TFs Orthodenticle (Otd), Spalt (Sal), and Spineless (Ss) regulate *dve* to yield a unique and fine-tuned pattern of high expression in outer PRs and weak expression in yR7s, a subset of inner PRs. We generated *dve* reporter gene constructs that cover the *dve* gene locus and found two constructs that exhibited strong GFP expression. One construct expressed GFP only in outer PRs, while a second construct drove expression in yR7s. We found that *dve outers>GFP* is activated by Otd and repressed by Sal, while *dve yR7>GFP* is activated by Ss and Sal in combination. Both *cis*-regulatory elements were repressed by Dve, suggesting the role of negative autoregulation in the governing of *dve* expression. Furthermore, we observed GFP expression at distinct stages of fly development (larva, pupa, and adult). Expression of *dve outers>GFP* was first seen in the pupa stage and remained consistent in the adult stage, while *dve yR7>GFP* was activated the larva stage, consistently expressed in the pupa, but ectopically expressed in all R7s in the adult. We hypothesize that long-range interactions of *cis*-regulatory elements may control *dve* expression and combining the two elements could yield the pattern observed in wildtype. Ectopic R7 expression could not be rescued when the elements were expressed in *trans*, and our next step is to observe expression with the elements in *cis*. Understanding the mechanisms behind *dve* regulation may provide insight into sensory system development in Drosophila and other species sharing similar regulatory networks.

831B

Flexibility and Constraints of Hox complex configuration on Drosophila cis-regulatory modules. Arya Zandvakili, Juli Uhl, Ian Campbell, Yi Kuang, Brian Gebelein. Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

There has been a long-standing question in molecular biology of how transcription factors (TFs) bind and function only at a subset of DNA sequences - called cis-regulatory modules (CRMs). The *Drosophila* Hox factor, Abdominal-A (Abd-A), along with its co-factors Extradenticle (Exd) and Homothorax (Hth), can mediate both activation and repression when bound to different CRMs. Two such CRMs are *RhoA*, which activates *rhomboid* transcription to promote EGF secretion from a subset of abdominal cells, and *DCRE*, which represses *distalless* transcription to prevent leg development in the abdomen. Although *RhoA* and *DCRE* can both be bound by Abd-A/Exd/Hth, they differ in the arrangement and number of binding sites for these factors. Furthermore these CRMs require binding of additional factors for functionality - namely *RhoA* requires binding of Pax2 to function as an activator and *DCRE* requires binding of Slp2 to function as a repressor. In this study, we use transgenic reporter assays to test how TF binding site configuration contributes to the opposing functions of these two CRMs. We show that the repressive function of *DCRE* is maintained when the Abd-A/Exd/Hth site of *DCRE* is replaced with that of *RhoA*. In contrast, the Abd-A/Exd/Hth site of *DCRE* does not appear to be functional in the context of *RhoA*. The relative positioning and orientation of Hox with either Slp2 or Pax2 appears to be critical for the function of both CRMs. These data indicate that in a single CRM there is flexibility in the arrangement of certain TF binding sites, but constraint in others. Future directions of this study include deciphering the mechanisms underlying the flexibility of Hox/Exd/Hth configuration and the constraints on the configurations of Hox with either Pax2 or Slp2. Ultimately, understanding the patterns of TF configuration on CRMs may have application in predictive algorithms of TF binding and function. .

832C

Uncovering mechanisms that regulate miRNA expression. Victoria Church, Sigal Kellman-Pressman, Richard Carthew. Northwestern University, Evanston, IL.

MiRNA biogenesis begins with the transcription of a primary miRNA (pri-miRNA) transcript by RNA polymerase II (RPol II). Thereafter, the Microprocessor complex (composed of the RNase III enzyme Drosha and its cofactor Pasha) cleaves the pri-miRNA to form a pre-miRNA in the nucleus. The pre-miRNA is subsequently shuttled to the cytoplasm and delivered to Dicer, which then processes the pre-miRNA into a mature-miRNA that is loaded into the RISC complex for gene silencing. While we understand the mechanisms that control

production of mature miRNAs, we do not understand the mechanisms that control the specificity of miRNA expression. What factors promote or inhibit Drosha and Dicer in a way that allows for expression of specific miRNAs in one cell type and not another, or at an exact developmental time? A forward genetic screen performed in our lab recovered a mutant allele of a gene previously unknown to participate in the miRNA pathway. A GFP reporter with binding sites for endogenous miRNAs in its 3'UTR was de-repressed in the mutant tissue, and a genomic transgene rescued the defect. The reporter de-repression required the miRNA binding sites in its 3'UTR, indicating a defect in miRNA activity in the mutant. Northern blot and qPCR analyses revealed a decreased level of mature miR-7 in homozygous mutant eye tissue compared to control tissue, with no difference however in primary miR-7 level, indicating a defect downstream of transcription of the primary miR-7 gene. Of 100 mature miRNAs detected by deep-sequencing, 18 miRNAs (including miR-7) were down-regulated in the mutants when compared to the control. Interestingly, 34 miRNAs were up-regulated. We believe that this factor is bi-directionally regulating miRNA expression in cells by promoting or inhibiting processing of specific miRNAs via Drosha or Dicer. .

833A

An in vitro study of Zelda as a pioneer factor. H. Crimmins, C. Rushlow, N. Kirov. Department of Biology, New York University, New York, NY.

The zinc finger transcription factor Zelda plays an essential role in the activation of many Drosophila pre-blastoderm genes. Zelda works together with the patterning transcription factors to activate many downstream targets. It can be detected in interphase nuclei in very young embryos, before the patterning factors. In addition, genome-wide ChIP assays found that Zelda associates early on with known target enhancers, suggesting a role as a pioneer factor that can interact with chromatin prior to other factor binding, thus establishing transcriptional competence. Here we investigate the ability of Zelda to interact with chromatin. We use in vitro reconstituted chromatin templates to study Zelda binding to its cognate sites wrapped in nucleosomes, and its effect on accessibility of other transcription factors to their binding sites on the same chromatin templates. Our preliminary results show that Zelda is able to bind to nucleosomal templates under certain conditions, dynamically competing with histones.

834B

Hypergravity-induced transcriptome profiling in *Drosophila* pupae via deep mRNA sequencing. Ravikumar Hosamani[1], Shannon Hateley[2], Lior Pachter[2], Sharmila Bhattacharya[1]. 1) Space Bioscience Division, NASA Ames Research Center, Mountain View, CA; 2) Department of Molecular and Cell Biology, University of California, Berkeley.

Altered gravity has a profound influence on gene expression, which in turn affects the development, structure, and function of an organism. In this study, we used deep mRNA-Seq to profile chronic hypergravity (3G, three x earth's gravitational force)-induced changes in the transcriptome of early pupae. Canton S eggs were laid, allowed to hatch, and attain early pupal stage while exposed to hypergravity. Following hypergravity exposure, total RNA was extracted from these pupae, and samples were processed using the Illumina TruSeq mRNA kit. Multiplexed libraries were sequenced on the HiSeq2000, and reads were mapped to the *D.melanogaster* transcriptome using Bowtie2. Transcript abundances were quantified with eXpress, and gene-level differential expression analysis was performed using the R package DESeq.Transcriptome data revealed a significant up-regulation of *Attacin D* (3.6 fold) and *Turandot A* (5.6 fold) genes in the hypergravity condition, suggesting altered immune function and humoral stress response, respectively. Another set of genes that exhibited increased expression in response to hypergravity were *Notopleural, CG8172, CG8170,* and *CG8213,* which have serine-type endopeptidase activity. Heightened expression of these genes may indicate increased proteolysis in hypergravity-treated pupae. *Dusky*, a gene involved in imaginal disc-derived wing morphogenesis, was increased (4.4 fold) in the hypergravity condition, implying that altered gravity has a direct impact on specific developmental processes. Interestingly, a robust increase in mRNA level (33 fold) of *CG5756* in hypergravity suggests altered chitin metabolism, and eventual affect upon downstream, chitin-based cuticle development in pupae. GO analysis revealed that genes related to lateral inhibition *(LSP 1 beta)*, circadian rhythm *(Early gene at 23)*, lipid metabolism *(CG6431)*, and alcohol metabolism *(CG45065, CG45064)* were also significantly affected in hypergravity. Collectively, mRNA sequencing data reveals that chronic hypergravity affects diverse physiological processes in *Drosophila* pupae.

835C

Gene expression analysis of known CASK interacting proteins in CASK knockout fly lines. Alexander Kneubehl, Rachel Boody, Lauren Guerriero, Jamie Sanford. Ohio Northern University, 525 S. Main Street, Ada, OH 45810.

Calcium/calmodulin-dependent serine protein kinase (CASK) belongs to the membrane-associated guanylate kinases (MAGUK) protein family. CASK is most highly expressed in the central nervous system across multiple species, where it is known play both pre- and post-synaptic roles in the formation of learning memory. Interestingly, *Drosophila* CASK (dCASK) is also highly expressed in ovaries and is implicated in border cell migration during *Drosophila* oogenesis. Currently, there are two CASK knockout fly lines utilized in analyses of dCASK gene function, the $CASK^{307/313}$ (x-ray mutagenesis) and the p18 (p-element insertion) fly lines. The goal of the present study was to determine whether CASK gene knockout alters the gene expression of its known protein interactors: Discs Large 1 (dlg 1), veli, X11L/Mint and Grip. These genes are known to form various tripartite complexes with CASK, and a CASK – Veli - X11L/Mint complex mediates vulval induction in *C. elegans*. Reverse-Transcriptase (RT)-PCR was conducted on whole fly extracts from w1118, $CASK^{307/313}$ transheterozygote and p18 knockout fly lines using gene specific primers for dlg1, veli, X11L/Mint and Grip. Gene expression results quantitated by NIH Image J analysis indicate that there are no significant changes in gene expression across fly lines lacking expression of the CASK gene. These results demonstrate that CASK knockout does not have a corresponding affect on the gene

expression of its known interactors in whole fly analysis. However, tissue-specific analysis still warrants further investigation. As little work has been done to characterize CASK's putative role in oogenesis, future work will focus on tissue-specific gene expression and protein localization analysis of dlg1, veli, X11L/Mint and Grip in both wild-type and CASK knockout ovaries. These investigations present an initial step toward determining whether the tripartite complex known to function in *C. elegans* vulva development is evolutionarily conserved in *Drosophila* ovary development. .

836A

Regulation of the Master Regulatory Helix-Loop-Helix Proteins in *Drosophila*. Ke Li, Nicholas Baker. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

E (Enhancer) proteins and ID (Inhibitor of DNA binding/Differentiation) proteins are broadly expressed, master regulators of tissue-specific basic HLH (bHLH) proteins, whose functions are critical in neurogenesis, myogenesis and many other developmental processes. Disruption of E- and ID protein functions is observed in many cancers and neurocognitive disorders. Bhattacharya and Baker (2011) reported that Daughterless (Da) and Extra macrochaetae (Emc), respectively the sole E- and ID protein in *Drosophila*, control one another's expression, and identified a transcriptional enhancer in the *da*gene that mediates autoregulation. By *in situ* hybridization, we found Da regulates *emc* both transcriptionally and post-transcriptionally, and is the dominant determinant of Emc protein expression. The mechanisms of post-transcriptional regulation are also under investigation. To address the regulation of *emc*, we used genomic rescue to map its regulatory sequences. We identified two functionally redundant regulatory elements in the proximity of the *emc* transcription locus. Contrary to previous conclusions, we also show that the proneural bHLH protein Atonal regulates *da* and *emc* expression in the morphogenetic furrow of the eye disc. Since *da* and *emc* are regulated by Atonal, all aspects of this proneural region are controlled by the main proneural gene.

837B

miR-184 regulates the level of Gliotactin, a tricellular marker, through BMP signaling. Zohreh Sharifkhodaei[1], Mary Gilbert[1], Mojgan Padash-Barmchi[1], Gayathri Samarasekera[1], Tudor A Fulga[2], David Van Vactor[2], Vanessa Auld[1]. 1) Department of Zoology, University of British Columbia, Vancouver, Canada; 2) Department of Cell Biology, Harvard Medical School, Boston MA, USA.

Permeability barriers and maintaining the integrity of the epithelial barriers created by bicellular and tricellular junctions are essential for animal viability. The tricellular junction (TCJ) is formed at the convergence of three cells and their septate junctions. The TCJ forms a permeability barrier at the corner of cells and is critical in blocking the flow of fluids or pathogens between the gaps created at the corners of cells. Gliotactin, a single-pass transmembrane protein, is the only known component of the Drosophila tricellular junction and is critical for the formation of the septate junction and permeability barrier function. Gliotactin mutants disrupt septate junction formation, maturation, and tricellular junction function. Gliotactin levels are tightly controlled with tyrosine phosphorylation and endocytosis controlling localization to the tricellular junction. Using the UAS/Gal4 system in the wing imaginal disc, we show that Gliotactin is also regulated at the mRNA level. Specifically we found that miR-184 mediates Gli mRNA degradation through highly conserved target sites in the 3'UTR. miR-184 also controlled a suite of other septate junction proteins such as Neurexin-IV, coracle and macroglobulin complement-related. Overexpression of Gliotactin triggered the expression of miR-184. The induction of miR-184 by Gliotactin occurred through thick-vein (Tkv), a BMP type-I receptor, and lead to phosphorylation and activation of the downstream transcription factor Mad. When the up regulation of miR-184 was blocked using a miR-184 sponge or by blocking Mad signaling, Gliotactin was no longer downregulated. We conclude that overexpression of Gliotactin activates the Tkv receptor to enhance the biogenesis and expression of miR-184 to control Gliotactin in conjunction with a core of other septate junction proteins.

838C

Stochastic *spineless* expression in natural *Drosophila* variants. Cyrus Zhou[1], Annie Cho[1], Haziq Siddiqi[1], India Reiss[1], Caitlin Anderson[1], Cameron Avelis[2], Elijah Roberts[2], Robert Johnston[1]. 1) Department of Biology, Johns Hopkins, Baltimore, MD; 2) Department of Biophysics, Johns Hopkins, Baltimore, MD.

Development sometimes utilizes stochastic mechanisms to diversify cell fates, in particular, in sensory systems. For example, in the human vision system, each color-detecting cone cell stochastically chooses to express one of three opsins that detect red, green, or blue light. Failure of proper stochastic gene regulation in human cones leads to disorders such as color blindness. Similar to the human cone cells, the Drosophila retina displays stochastic cell fate specification in its color vision system. The R7 photoreceptors randomly decide to express either Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). This decision is governed by the stochastic expression of the transcription factor Spineless (Ss). When Ss is on, it induces Rh4 expression and Rh3 repression. When Ss is off, Rh3 is expressed and Rh4 is absent. In wild-type lab-stocks, approximately 65% of R7 cells express Rh4 (Ss on). However, the 200 wild-type Drosophila Genetic Reference Panel (DGRP) lines display a huge range of expression in Ss expression, from 20% to 80%. Our GWAS analysis on these lines has identified two SNPs in the *spineless* (*ss*) locus that putatively causes a change in *ss* expression frequency, one of which causes high *ss* expression (*high SNP*) and the other causes low *ss* expression (*low SNP*). To identify more SNPs specifically in spineless, we crossed the DGRP lines to a *ss* deficiency stock and looked at the frequency of *ss* expression in the progeny. These progeny were hemizygous for *ss*, but heterozygous with lab stock for all other genes. Excluding epistatic SNPs, this allows us to analyze solely the effect of SNPs in the *ss* locus on *ss* expression levels. Upon completing our assessment of the 200 DGRP lines, we will use GWAS and look for causative SNPs in the *ss locus*. Currently, our data confirms our previous finding with the *high SNP* and *low SNP*. We have shown that GWAS on the DGRP lines was an effective method of locating ss-affecting SNPs. GWAS on our current dataset will determine additional SNPs that are located in the *ss* locus.

839A

How does Ago2 contribute to dosage compensation in *Drosophila melanogaster*? Nikita Deshpande, Victoria Meller. Wayne State University, Detroit, MI.

Dosage compensation is an essential process that adjusts expression of X-linked genes to equalize their expression in males and females. Drosophila melanogaster males achieve dosage compensation by transcribing X-linked genes at twice the rate that females do. The Male Specific Lethal (MSL) complex binds X-linked genes in males and modifies chromatin to increase expression. The mechanism that restricts this complex to X chromatin is unknown. Our lab has found that the siRNA pathway, as well as siRNAs produced from the X chromosome, participate in dosage compensation in flies. Interestingly, the siRNA pathway contributes to X-localization of the MSL complex. The basis of enhanced localization is unknown, and no RNAi components have been found to interact directly with the MSL complex. This suggests that the siRNA pathway influences X-recognition by a novel mechanism. For example, Ago2-containing complexes could bind nascent RNAs from the X chromosome, thus localizing activities that alter chromatin structure. An X-specific chromosome conformation might facilitate MSL recruitment and spreading into X chromatin. To test this model, we performed a targeted screen of genes known to interact with Ago2. This identified several genes that contribute to dosage compensation. We are examining the role of these genes in modification of X-linked chromatin. We are also testing direct binding of Ago2, and key interacting proteins, to siRNA-producing regions of the X chromosome. By resolving the molecular mechanism by which Ago2 contributes to X-recognition this study will serve as a model for how small RNA contributes to coordinated regulation of broad chromosomal domains in eukaryotes.

840B

Identification and Characterization of a Novel Regulator of Interphase Chromosome Structure. Michael Nash, Nina Hann, Barbara Fasulo, Stephanie McClymont, John Tamkun. Molecular, Cell, & Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA.

The packaging of DNA into chromatin is critical for the organization and expression of eukaryotic genes. Nucleosomes, the fundamental unit of chromatin structure, repress transcription by blocking the binding of transcription factors and other proteins to DNA. Chromatin organization above the level of the nucleosome, including chromosome pairing, folding and looping also modulate transcription, but the mechanisms underlying the regulation of higher-order chromatin structure remain poorly understood. To identify novel factors involved in the regulation of higher-order chromatin structure, we screened for X-linked, EMS-induced mutations that disrupt the organization of *Drosophila* salivary gland polytene chromosomes. One of the mutations recovered in this screen, 1121, is a loss of function mutation that dramatically alters polytene chromosome structure. The male X chromosome of 1121 mutant larvae appears decondensed and its banding pattern is grossly abnormal. Less striking defects in the structure of the autosomes were also observed in 1121 mutants. Live analysis and confocal microscopy revealed that the 1121 mutation disrupts the binding activity of the MSL3 subunit of the dosage compensation complex, causing it to form a diffuse cloud around the male X chromosome. The 1121 mutation was mapped to a relatively small region of the X chromosome (10B3-10B5) lacking any genes previously implicated in dosage compensation. Our findings suggest that the 1121 mutation defines a novel gene required for both the stable association of the dosage compensation complex with the male X chromosome and the maintenance of higher-order chromatin structure.

841C

Zelda facilitates chromatin accessibility during the earliest stages of development. Katharine Schulz[1], Eliana Bondra[1], Jason Lieb[2], Tommy Kaplan[3], Daniel McKay[4], Melissa Harrison[1]. 1) Biomolecular Chemistry, University of Wisconsin Madison, Madison, WI; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) School of Computer Science and Engineering, The Hebrew University of Jerusalem, Israel; 4) Department of Biology, The University of North Carolina, Chapel Hill, NC.

In metazoans, the initial stages of development are driven by maternally supplied RNAs and proteins while the zygotic genome remains transcriptionally silent. Ultimately, developmental control is transferred to the zygote through a process known as the maternal-to-zygotic transition (MZT). Through this transition a totipotent state is established and the zygotic genome is poised for widespread activation. In *Drosophila* the transcription factor Zelda (ZLD) plays an essential role in poising the genome during the MZT, yet, the mechanism by which this protein acts remains unknown. We have previously shown that ZLD binding is associated with chromatin accessibility at the MZT. To determine if ZLD is instrumental in establishing or maintaining this open chromatin, we used Formaldeyde Assisted Isolation of Regulatory Elements (FAIRE) to compare chromatin accessibility in the presence and absence of maternally deposited ZLD. We identified a decrease in chromatin accessibility specifically at a subset of loci normally bound by ZLD, demonstrating that ZLD binding shapes the chromatin landscape of the early embryo. Genomic regions that are less accessible in the absence of ZLD are enriched in introns and intergenic regions associated with genes that depend on ZLD for transcriptional activation. This suggests that the ability of ZLD to facilitate chromatin accessibility is important for transcriptional activation. Based on these data, we propose that ZLD acts as a pioneer factor to define regions of open chromatin at the MZT, granting transcriptional regulators access to specific loci and allowing widespread transcriptional activation. .

842A

Do the physiological effects of exercise depend on genetic background. Louis Watanabe, Nicole Riddle. Biology, University of Alabama at Birmingham, Birmingham, AL.

Regular exercise and moderate activity levels are considered important components in maintaining a healthy life-style; however, the

impact of genetic variation on the physiological effects of exercise is poorly understood. Experiments in humans suggest that genetic background influences the effects of exercise on metabolism, and studies in mice have shown that genetic background is indeed a significant factor in the effects of exercise on body composition. It is still unclear, however, what the interactions are between genetic background and exercise. *Drosophila melanogaster* is an ideal model organism for studying the genetics of exercise, because many of the relevant metabolic pathways are highly conserved. We use a novel combination of techniques to obtain data on the effects of exercise on lifespan, metabolism, and its associated chromatin changes using a fly exercise machine based on an original schematic developed at the University of Alabama by S. Mendez and L. Reed. This treadwheel exploits the inherent negative geotaxis of Drosophila and slowly rotates vials. Flies from the Drosophila Genetics Reference Panel 2, a fully genotyped collection of 200 inbred lines developed by Dr. Trudy Mackay, will be exercised on the treadwheel. Lifespan will be measured through daily survival counts, and we hypothesize that exercise is beneficial to lifespan, but that the effect varies based on genetic background. Metabolism will be measured through O2k analysis and mitochondrial counts. We suspect that exercise will increase metabolic capacity, but that again there will be variation based on genotype. Chromatin changes will be elucidated through ChIP analysis, and we suspect there will be differences in modifications between the genotypes. These experiments will also provide researchers studying exercise in other model organisms novel insights for treating exercise related diseases such as obesity and diabetes. These studies will ultimately help to elucidate how and why different genetic backgrounds cause exercise to have different effects on *Drosophila*..

843B

Precise induction of ectopic centromeres in *Drosophila* as a model for neocentromere formation. Jason Palladino, Barbara Mellone. Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT.

Neocentromeres are functional "new" centromeres that form at non-centromeric locations and are associated with cancer progression, birth defects and the evolution of new karyotypes in mammals. The spontaneous formation of neocentromeres is rare, making the study of their establishment and impact on cell and organism viability unfeasible. We have generated a line of *Drosophila* that form ectopic centromeres at defined genomic locations via chromosomal tethering of CAL1, the *Drosophila*-specific CENP-A assembly factor, through the *lacO*/LacI targeting system. We report the impact of the presence of a single ectopic centromere in each cell on animal viability, genome stability, tissue and cellular function. This unique animal model will allow the study of neocentromere establishment, maturation and, possibly, suppression, and of the potential of neocentromeres to induce tumorigenesis.

844C

The impact of ectopic piRNAs on hetrochromatin assembly at a PEV reporter site. Kiri Ulmschneider, Sarah C.R. Elgin. Dept Biol, Washington University, St. Louis, MO.

Transposable elements (TEs) and their remnants make up a large part of eukaryotic genomes, forcing eukaryotes to use a variety of mechanisms to control TEs and protect their genomes. In Drosophila, the piRNA system acts in the female germline and early embryo to repress transposons, but much about how this system functions is not known. Although part of the piRNA pathway appears to work through post-transcriptional silencing, as is the case for other RNAi systems, the Argonaute protein Piwi requires nuclear localization to silence transposons. We have previously identified chromatin sites where the presence of a TE results in variegation of an adjacent *hsp70-white* reporter, similar in appearance to Position Effect Variegation (PEV). This effect disappears and one observes a full red eye when the TE is excised from the site. Mutations in the piRNA pathway also cause a loss of silencing. Using this reporter site, we are testing what DNA elements are necessary for silencing to be targeted to these loci, in particular testing elements found in piRNA populations. We are testing DNA homologous to piRNAs from the original transposon and from additional transposons that show overexpression when there is a loss of function in the female germline piRNA pathway. Variegation of the *hsp70-white* reporter, quantified by pigment assays, will show whether or not these ectopic piRNAS are sufficient to induce silencing at these sites. Additionally, read-through transcription at the reporter site occurs, and may be necessary for silencing. This would suggest an RNA-RNA pairing mechanism for targeting silencing. We will test this hypothesis by using a transposon flanked by transcription termination elements; a lack of silencing would indicate that transcription of the transposon is necessary for silencing. We hope to learn more about what specific elements are necessary for triggering silencing by the piRNA pathway.

845A

New Evidence for Piwi Association with Specific Sites in the *Drosophila* Genome. N. Neuenkirchen, N. Liu, M. Zhong, H. Lin. Stem Cell Center and Department of Cell Biology, Yale University, New Haven, CT.

How epigenetic factors without DNA binding ability are recruited to specific sites in the genome represents a key question in epigenetic programming. *Drosophila* Piwi is one of the three members of the PIWI family that bind to short non-coding RNAs of 24-31 nt in length. Piwi localizes to the nucleus and regulates a wide range of processes including germline stem cell maintenance and self-renewal, transposon repression and epigenetic regulation. Recently, Piwi has been shown by chromatin-immunoprecipitation and deep sequencing (ChIP-seq) to be guided to distinct genomic regions by their associated piRNAs. However, the experimental challenges that accompany this approach make it difficult to corroborate these findings by the research community. Here we present an independent approach to study the interaction of Piwi with chromatin using DamID-seq (DNA adenine methylation ID and deep sequencing). Comparing the random methylation of N6 in the adenine of GATC sites by Dam (DNA adenine methyltransferase) and the Piwi-guided methylation by the Dam-Piwi fusion protein, our approach covers 78.45% of the genomic GATC sites. We find that Piwi-specific methylation occurs predominantly in euchromatic regions, indicating specific association of Piwi with these regions. Heterochromatic regions are targeted less frequently, likely resulting from their inaccessible chromatin structure. Furthermore, we show that the number

of piRNA complementary sites in close proximity to methylated GATC sites is significantly enriched for Piwi-specific methylation. These findings support the association of Piwi with chromatin.

846B

Chromatin remodelers, nucleoplasm compartment and proteinopathies. Luca Lo Piccolo, Rosa Bonaccorso, Antonia Maria Rita Ingrassia, **Maria Cristina Onorati**. STEBICEF Dept, viale delle Scienze Edificio 16, Universita' degli Studi di Palermo, 90128 Palermo.

Recent advances in the field of transcriptome exploration have revealed novel sets of new ncRNAs like the long non-coding RNAs, which seem to be key components of epigenetic regulatory networks. Indeed, recent studies have shown that lncRNAs regulate the gene expression by chromatin remodelling, transcription, splicing and RNA decay control, enhancer function, and epigenetic regulation. An emerging theme from multiple model systems is that lncRNAs form extensive networks of ribonucleoprotein (RNP) complexes with numerous chromatin regulators and then target these enzymatic activities to appropriate locations in the genome. Using *D. melanogaster* as model system, I recently found a functional interaction between ISWI, the catalytic subunit of several ATP-dependent chromatin-remodeling complexes, and the lncRNA hsr-omega (hsrω). In *Drosophila* the nucleus-limited hsrω-n transcript is dynamically associated with several different heterogeneous nuclear ribonucleoprotein (hnRNPs) in the nucleus to organize the omega speckles. Omega speckles, play essential roles in the regulation of RNA processing reactions and their alteration could promote aberrant expression of hnRNPs with severe change in various mRNA expression and processing of their targets genes. Indeed, emerging neurodegenerative diseases as proteinopathies, seem to be caused by alteration in hnRNPs nucleus amount and localization. I have evidence that the hnRNP TBPH, the *Drosophila* homolog of human TAR DNA binding protein (TDP-43 or TARDBP), the major protein present in cytoplasmic inclusions in neurodiseases, is a component of the omega speckles. Interestingly, I have data strongly suggesting that ISWI is involved in the regulation of correct localization and functioning of TBPH protein. I report preliminary results showing in *D. melanogaster* the role of of chromatin remodelers in nucleoplasm compartment organization and their possible role in proteinopathies onset and development.

847C

The effects of knocking down the expression of TnaA, a trithorax group protein with a putative SUMO E3 ligase function in *Drosophila melanogaster*. Marco Rosales-Vega, Zoraya Palomero, Mario Zurita, Martha Vázquez. Univ. Nacional Autónoma de México. Cuernavaca, Morelos. México.

Homeotic (Hox) genes specify the identity of body segments. Two groups of proteins are required for maintenance of Hox gene expression. Polycomb-group (PcG) proteins maintain Hox repression whereas trithorax-group proteins (trxG) maintain Hox activation. *tonalli (tna)* is a trxG gene identified because of its genetic interaction with *brahma* (*brm*). *brm* encodes the ATPase of BAP and PBAP complexes in Drosophila. *tna* encodes at least one protein isoform called TnaA that contains a SP-RING Zn-finger domain characteristic of SUMO E3 ligases. Besides *brm*, *tna* interacts genetically with other subunits of BAP and/or PBAP chromatin remodeling complexes such as *osa* and *moira*, with *kismet*, the ATPase of a chromatin remodeling factor required for elongation, and with *skuld* a subunit of the Mediator complex. In this work we designed an RNAi-based strategy to knockdown TnaA expression levels. We will show that ubiquitous *tna* RNAi expression, or directed-expression to specific organs, reduces the levels of TnaA in adults, in whole third instar larvae or specifically in larval salivary glands. Furthermore, ubiquitous TnaA knockdown causes lethality at late pupal stages in a temperature-dependent manner, and the lethality is greater in males than in females. Some of the flies that die at early pupal stages show head eversion defects. The survivor adult females expressing ubiquitously *tna*RNAi display a reduced longevity. We will show that when TnaA levels are diminished in the halteres, a partial homeotic transformation to wings is observed, similar to the phenotype observed when SUMO is knocked down in the halteres (Smith *et al*., 2011). TnaA and Osa colocalize at discrete sites on polytene chromosomes from salivary glands of wild-type third instar larvae, suggesting a role of TnaA on the proper function of BAP and PBAP complexes. One possibility is that proper BAP and PBAP assembly or recruitment to chromatin depends on TnaA-mediated SUMOylation. In this work we will show co-immunolocalization of TnaA and Osa in *tna* defective polytene chromosomes of third instar larvae.

848A

The newly identified HMG-box proteins tHMG-1 and tHMG-2 interact during chromatin remodeling in *Drosophila* spermatogenesis. Silke Rothenbusch, Stefanie M. K. Gärtner, Ina Theofel, Christina Rathke, Renate Renkawitz-Pohl. Philipps University, Marburg, Hessen, Germany.

The process called spermatogenesis is highly conserved throughout the animal kingdom. Production of mature spermatozoa in the male germ line of many animal species is characterized by remodeling chromatin from histone-based nucleosomes to protamine-based structures. This is best studied in mammals and *Drosophila* . The remodeling effects condensation of sperm chromatin, producing highly compact chromatin in mature spermatozoa. During histone to protamine transition, several chromatin binding proteins presumably act together in chromatin remodeling. One family of chromatin binding proteins represents the HMG box protein family, whose members are characterized by a DNA binding domain called HMG box. HMG box proteins like Tpl^{94D} and the protamines are expressed at the nuclei during spermiogenesis in *Drosophila*. They may be involved in chromatin remodeling processes by directly altering chromatin structures or by interacting with chromatin remodeling complexes. Since males, where both protamine genes are deleted, are still fertile and show motile sperm, we assume there must be further proteins with the ability to bind chromatin to secure this important process. Recently, we identified tHMG1 and tHMG2 as testis specific HMG box proteins exhibiting a nuclear distribution pattern. Interaction studies using ectopic expression of tHMG1 and tHMG2 in *Drosophila* larval salivary glands indicate that the HMG box proteins can act as

heterodimers and homodimers. Data suggest that tHMG1 and tHMG2 interact during spermiogenesis in the canoe stage, where histones are removed from chromatin and protamines are loaded, thus at the time when Tpl^{94D} is transiently expressed. Even so, mutant flies lacking both proteins exhibit no obvious alterations in spermatogenesis. These results indicate a high redundancy in spermiogenic chromatin remodeling. Indeed, transcription of the somatic homologue HMGZ is 2-3 fold upregulated upon loss of tHMG1 and tHMG2. Therefore, future work will include the identification of further proteins, which act during spermiogenesis to promote chromatin remodeling in *Drosophila*.

849B

The dAdd1 proteins: The *Drosophila* orthologs of the amino-terminus of the ATRX vertebrate protein. Viviana Valadez[1], Brenda López[1], Silvia Meyer[1], Benjamín Hernández[1], Daniel Montero[1], Adam Campos[2], Enrique Rudiño[2], Martha Vazquez[1], Mario Zurita[1]. 1) Genetica del Desarrollo, Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; 2) Medicina Molecular y Bioprocesos. Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, México.

The human *ATRX* gene encodes a chromatin remodeling protein that has two important domains, an helicase/ATPase domain and a domain composed of two zinc fingers called the ADD domain. The ADD domain binds to histone tails and has been proposed to mediate hATRX binding to chromatin. The putative ATRX homolog in *Drosophila (datrx*) has a conserved helicase/ATPase domain but lacks the ADD domain. We performed a bioinformatic search in the data bank of the *Drosophila* genome and found a gene (recently named *dadd1*) that encodes three proteins that share a common region in the amino terminal end that contains an ADD domain highly similar to the ADD domain of the hATRX protein. 3D modeling of the domain shows that the structure and aminoacids which mediate the histone tail contacts are highly conserved. The three protein isoforms are generated by alternative splicing and are expressed throughout the development of *D. melanogaster*. We determined using pull-down and CoIP assays that they interact physically with $dATRX_L$ and HP1a. Furthermore co-immunostaining of polytene chromosomes with specific antibodies show that they co-localize mainly in the chromocenter, with $dATRX_L$ and HP1a, although euchromatic localization can also be seen through the chromosome arms. ChIP experiments demonstrate that these proteins are present *in vivo* in the same heterochromatic regions. Flies carrying combinations of *datrx* and *dadd1* alleles result in the generation of melanotic masses in the organism. Phylogenetic inference analysis shows that in the Insecta class the *ATRX* gene suffered a fission event. Our data strongly support that the *dadd1* encoded proteins participate with dATRX in some cellular functions such as heterochromatin maintenance and that they represent the possible orthologs of the amino-terminal domain of hATRX.

850C

Interactions between the Myb Oncoprotein and the Variant Histone H2A.V in the *D.melanogaster* Hematopoietic Organ. M. Grigorian[1], H. DeBruhl[2], J. Lipsick[1]. 1) Depts. of Pathology and Genetics, Stanford University, Stanford, Ca; 2) Dept. of Mol., Cell and Dev. Biology, University of California, Santa Cruz, Santa Cruz, Ca.

The dREAM/Myb-MuvB complex (Drosophila Rb, E2F, and Myb-associated proteins) is a key player in maintaining control of genes involved in regulation of cell cycle progression and differentiation. The complex is composed of Myb, E2F2, DP, Mip120, Mip130, Mip40, Caf1/p55, Lin52 and either RBF1 or RBF2. The complex is generally repressive in function. Myb counteracts this repression and was first identified as an oncogene in a chicken leukemia virus. It has since also been linked to the activation of a number of genes important for chromosome condensation, spindle assembly, and chromosome segregation. Recent work in our lab has shown that Myb null mutants display an accumulation of binucleate cells in larval wing discs due to defective cytokinesis. Interestingly, this binucleate phenotype can be repressed if a heterozygous Histone 2A Variant (H2A.V) mutation is crossed into the Myb null background. This suggests a requirement for H2A.V in the dREAM complex's repressive role. H2A.V is a histone variant that can replace the canonical histone H2A in chromatin. Vertebrates have multiple H2A variant histones, including H2A.Z and H2A.X. H2A.Z is linked to transcriptional activation as well as maintenance of gene silencing, while H2A.X is important in DNA damage repair. Increased H2A.Z expression has been linked to cancer progression while loss of H2A.X has been linked to oncogenic transformation and increased chromosomal rearrangements. *D. melanogaster* has one histone H2A variant, H2A.V, which is a chimera of H2A.X and H2A.Z. Given the known role of the Myb oncogene in leukemia, we are utilizing the *D. melanogaster* hematopoietic system to study the interaction between Myb and H2A.V. Past studies from our lab have shown Myb null larvae have decreased levels of differentiated hemocytes. We have recently discovered that homozygous H2A.V mutant larvae display black melanotic tumors, likely arising from the larval hematopoietic organ. We therefore believe the utilization of this system will allow us to better understand the interaction of these two genes and their possible roles in cancer.

851A

An ancient centromeric histone duplication in *Drosophila*. Lisa E. Kursel[1,2], Harmit Malik[1,3]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington Molecular and Cellular Biology; 3) HHMI.

Centromeres are the chromosomal sites of kinetochore protein binding and microtubule attachment. Most eukaryotic organisms have epigenetically defined centromeres in which CenH3 replaces canonical H3 in the nucleosome particle. In *Drosophila,* CenH3 (called Cid) is both necessary and sufficient for centromere function. In line with the necessity for tight regulation of *CenH3* expression, nearly all eukaryotic genomes encode a single copy of *CenH3* despite containing many copies of canonical *H3*. We find that the *Drosophila* subgenus, which contains the virilis subgroup, has maintained a second putative centromeric histone gene, *Cid2*, for 40 to 50 million years. Cid2 localizes to centromeres in *D. virilis* cell culture. RT-PCR revealed that *Cid2* is expressed primarily in the male germline of *D. virilis*flies whereas ancestral *Cid* is ubiquitously expressed. Finally, we have used Cid2-specific antibodies to determine

that Cid2 functions post-meiosis and localizes to foci in the nuclei of developing spermatids in *D. virilis*. These results suggest that Cid2 has acquired a specialized centromeric role in the male germline. Our research will provide the first example of *in vivo* specialization of dual *CenH3s*, challenging the paradigm that all eukaryotes encode a single CenH3 or functionally redundant dual *CenH3s*.

852B

The Mof Acetyltransferase Is Required for JIL-1 H3S10 Kinase Stability in *Drosophila* Males. Yeran Li, Weili Cai, Chao Wang, Huai Deng, Xiaomin Bao, Weiguo Zhang, Jack Girton, Jorgen Johansen, Kristen Johansen. Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA.

Mof is a histone H4K16 acetyltransferase in both *Drosophila* and mammals. Mof, is a key component of the MSL (male specific lethal) dosage-compensation complex on the X-chromosome. However, Mof is also a member of the NSL (non-specific lethal) complex that is present in both sexes. The JIL-1 kinase localizes specifically to euchromatin interband regions of polytene chromosomes, is the kinase responsible for phosphorylation of histone H3S10 at interphase, and is up-regulated on the male X chromosome. However, it is not known how this localization is regulated or what causes JIL-1 to be enriched on the male X chromosome. To begin to address these issues and to study JIL-1's functional relationship to Mof we performed various combinations of double labelings of polytene chromosomes with JIL-1, Mof, H3S10ph, and H4K16ac antibodies. The results showed that JIL-1 and JIL-1 mediated H3S10 phosphorylation colocalize with Mof and H4K16 acetylation both on male and female chromosomes. We also performed RNAi-mediated knockdown of Mof using the Gal4-UAS system. Interestingly, we found that JIL-1 levels and localization were unaffected by Mof RNAi in females, but JIL-1 levels were substantially decreased in males. In contrast, loss of JIL-1 did not cause obvious changes to Mof or H4K16 acetylation localization or levels on polytene chromosomes. By analysis of a Mof partial loss-of-function mutant, *mof1*, we confirmed that a reduction of Mof acetyltransferase activity led to a substantial reduction of JIL-1 and H3S10 phosphorylation on polytene chromosomes in males. These levels could be restored by expression of Mof-LacI in the *mof1* mutant background indicating that the loss of JIL-1 indeed was caused by reduced Mof activity. Furthermore, applying a LacI tethering system we show that ectopic targeting of LacI-Mof induces enhanced H4K16 acetylation and recruitment of other MSL complex proteins, but not of JIL-1. Taken together, these results suggest that Mof acetyltransferase activity is necessary for stabilizing the JIL-1 kinase, but not for its chromosome localization in *Drosophila* males.

853C

Sequence-independent transcription is required for centromeric CENP-A deposition by its assembly factor. Chin-Chi Chen[1], Sarion Bowers[1], Zoltan Lipinszki[2,3], Jason Palladino[1], Emily Bettini[1], Sarah Trusiak[1], Leah Rosin[1], Marcin Przewloka[2], David Glover[2], Rachel O'Neill[1,4], **Barbara Mellone**[1,4]. 1) Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK; 3) Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, H-6701 Szeged, P.O. Box 521, Hungary; 4) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Centromeres are essential chromosomal structures that mediate accurate chromosome segregation during cell division. Centromeres are specified epigenetically by the heritable incorporation of the centromeric histone H3 variant, CENP-A. While many of the primary factors that mediate centromeric deposition of CENP-A are known, the chromatin and DNA requirements in this process have remained elusive. Here, we uncover an unexpected role for transcription in *Drosophila* CENP-A assembly. Using an inducible ectopic centromere system that uncouples CENP-A deposition from endogenous centromere function and cell-cycle progression, we demonstrate that CENP-A assembly by its loading factor, CAL1, requires RNA polymerase-mediated transcription of the underlying DNA. This sequence-independent transcription depends on the novel CAL1 binding partner FACT, but not on CENP-A incorporation. Our work establishes RNA polymerase passage as a key step in chaperone-mediated CENP-A chromatin propagation providing a new paradigm for the role of centromere transcription beyond that of centromeric RNAs.

854A

Tip60 mediated epigenetic control of cognition-linked genes. Priyalakshmi Panikker, Elefant Felice. Department of Biology, Drexel University, Philadelphia, PA.

Neurodegenerative disorders are often associated with defects in epigenetic gene control mechanisms that result in cognitive impairment. One of the most well characterized cognition linked epigenetic mark is histone acetylation that serves to control chromatin structure to mediate neuronal gene transcription profiles critical for cognitive ability. However, despite the importance of histone acetylation in higher order learning, the select cognition linked histone acetyltransferase (HAT) enzymes that generate these epigenetic marks remain largely unknown. We have shown that Tip60 HAT activity restores function in a number of cognition associated neuronal circuits negatively affected in an Alzheimer's disease (AD) Drosophila model. Our findings support a neuroprotective role for Tip60 under neurogeneration conditions. However, the mechanism by which Tip60 rescues these defects remains unclear. We hypothesize that Tip60 functions in neuroprotection by epigenetically reprogramming gene expression programs that work together to promote neuronal health and higher order brain function. We initially tested our hypothesis with a focus on cognition, by performing a pilot gene expression analysis screen on 15 Tip60 cognition linked targets that we identified as direct Tip60 gene targets in our Tip60 ChIP-Seq analysis. We crossed GAL4 responsive UAS-APP overexpression AD flies that we uniquely adapted to harbor a GAL4 responsive UAS-Tip60 wild-type ($Tip60^{WT}$) transgene to the $elav^{C155}$ pan-neuronal GAL4 driver to induce increased wild-type Tip60 HAT levels in the nervous system under APP induced AD neurodegenerative conditions. Control w^{1118} flies and AD flies overexpressing APP (UAS-APP) crossed to $elav^{C155}$-GAL4 served as base-line controls. We performed qPCR on cDNA isolated from staged 3rd instar larval brain tissue and found that while expression for all 15 genes tested was repressed in the APP flies, expression of 10 of these genes was restored in

the Tip60^{WT};APP flies. We are currently investigating mechanism(s) by which Tip60 positively reprograms such neuroprotective gene targets. Our findings should provide new insight into novel Tip60 HAT based neuroprotective mechanisms in neurological disorders. .

855B

Understanding Heterochromatin: Determination of DNA Elements That Influence Gene Expression. April Bauer, Sarah Elgin. Department of Biology, Washington University, St. Louis, MO.

The packaging of genomes into euchromatin and heterochromatin modulates gene expression in a dynamic process. Euchromatin is believed to be a transcriptionally accessible state, while heterochromatin formation blocks gene expression. Euchromatic genes juxtaposed with heterochromatin by rearrangement or transposition are silenced in some of the cells in which they would normally be active, resulting in a variegating phenotype (PEV). However, genes that are normally resident in constitutive heterochromatin are still appropriately expressed. The fourth chromosome of *Drosophila melanogaster*, a heterochromatic domain (based on criteria such as chromatin modification marks and a lack of recombination), provides an ideal model in which to study this dichotomy of gene expression in the context of a repressive environment. Our goal is to understand what features of fourth chromosome genes permit expression in the context of heterochromatin. We have identified a fourth chromosome MiMIC site wherein *hsp70-white*, a common reporter used to assess heterochromatin formation, exhibits PEV. We anticipate that a fourth chromosome gene will be fully expressed at this site, and hypothesize that there are unique DNA signatures associated with the gene that allow for expression despite the presence of inhibitory chromatin marks. Genes on the fourth often exhibit a characteristic chromatin pattern: the transcription start sites show an enrichment of H3K4me2/me3 and H3K9ac, whereas the body of the gene is enriched for the pericentric heterochromatin marks H3K9me2 and H3K9me3, as well as HP1a. We have chosen *Rad23*, a fourth chromosome heterochromatic gene (FHG) with characteristics typical of genes on the fourth, as a test gene for this purpose. Exchange of elements between *Rad23*and *hsp70-white* will illuminate how genes have adapted to their heterochromatic environment, by identifying which DNA elements promote appropriate gene expression on the fourth chromosome.

856C

Comparative Genomics of the Muller F Element among Four Species. John Braverman[1], Martin Burg[2], Christopher Jones[3], Nighat Kokan[4], Leming Zhou[5], Don Paetkau[6], Joyce Stamm[7], Christopher Shaffer[8], Wilson Leung[8], Sarah Elgin[8], other faculty and students of the Genomics Education Partnership. 1) Saint Joseph's University, Philadelphia, PA; 2) Grand Valley State University, Allendale, MI; 3) Moravian College, Bethlehem, PA; 4) Cardinal Stritch University, Milwaukee, WI; 5) University of Pittsburgh, Pittsburgh, PA; 6) St. Mary's College, St. Mary's City, MD; 7) University of Evansville, Evansville, IN; 8) Washington University, St. Louis, MO.

The *Drosophila melanogaster* Muller F element (or dot chromosome) has an estimated size of 4.2Mb, and the cytologically banded region of this element (~1.3Mb) contains approximately 80 protein-coding genes. This autosome is unusual in *D. melanogaster* because it is primarily heterochromatic, with a very low recombination rate. To understand how these properties impact evolution, faculty and students improved the public draft sequence and manually annotated the genes on the *D. erecta, D. mojavensis*, and *D. grimshawi* F elements and euchromatic reference regions from the Muller D elements. Comparative analysis of these annotations reveals that F elements of all four species maintained a higher repeat density than euchromatic reference regions. The *D. grimshawi* F element has the lowest transposon density. F element genes have larger coding spans, more coding exons, larger introns, and a lower codon bias compared to D element genes. Analysis shows that more of the codon bias in*D. grimshawi* F element genes can be attributed to selection than to mutational biases, suggesting that the density and types of transposons affect the degree of local heterochromatin formation. F element genes have lower estimated DNA melting temperatures than D element genes, potentially facilitating transcription through heterochromatin. Approximately 90% of the F element genes have remained on that element. The F element has smaller syntenic blocks than the genome averages, indicating higher rates of inversion despite lower rates of recombination. Overall, the F element has maintained characteristics that are distinct from those of other Drosophila autosomes, illuminating the constraints of a heterochromatic *milieu*.

857A

Investigation on the expansion of the *Drosophila ananassae* Muller F element. E. Chen, W. Leung, K. Ko, T. Quisenberry, S. Elgin. Washington University in St. Louis, Saint Louis, MO.

The distal arm of the *Drosophila melanogaster* Muller F element (~1.3Mb) has a gene density that is similar to other autosomes, but exhibits many heterochromatic characteristics, including high repeat density (~35%) and enrichment in HP1a and H3K9me3. The *D. ananassae* F element is unusual among the twelve genomes sequenced by the Drosophila 12 Genomes Consortium because it is substantially larger (~20Mb) than the F elements in the other species. A comparative analysis of the *D. melanogaster* and *D. ananassae* F elements could elucidate factors that contribute to the establishment and maintenance of heterochromatin, and regulation of gene expression in this domain. In order to determine the characteristics of this domain, undergraduate students participating in the Genomics Education Partnership (GEP) have manually improved the assembly of ~1.6 Mb and constructed gene models (~12 genes) for a portion of the *D. ananassae* F element and a euchromatic reference region derived from the Muller D element (~1.5Mb with ~110 genes). Analysis reveals a subset of the *D. ananassae* F element genes that have substantially larger introns (and larger coding spans) than their putative *D. melanogaster* orthologs. The increase in intron size can be attributed both to an increase in transposon density and to horizontal gene transfer from *Wolbachia* (an endosymbiont of Drosophila) into the *D. ananassae* genome. Analysis of the *Wolbachia* fragments found within the improved regions of the *D. ananassae* F element shows that some regions of the *Wolbachia* genome appear at a much higher frequency than others. Preliminary analysis suggests that many of

the *Wolbachia* fragments that have been integrated into the *D. ananassae* genome contain regions with sequence similarity to transposases and the *gag-pol* polyproteins from *Wolbachia*. Collectively, our analyses indicate that the horizontal gene transfer of *Wolbachia* has substantially altered the genomic landscape of the *D. ananassae* F element, including an increase both in total repeat density as well as in coding spans and intron sizes. Further studies are required to assess the functional impact of these *Wolbachia* fragments on the chromatin packaging and expression of *D. ananassae* F element genes.

858B

HP1B, A Member of the Heterochromatin Protein 1 Family. Tandy L Dolin Petrov, Nicole C Riddle. Biology, University of Alabama at Birmingham, Birmingham, AL.

HP1B is a member of the highly conserved, Heterochromatin Protein 1 (HP1) family of chromosomal proteins. The HP1 family of proteins has a variety of functions, including transcription regulation, DNA repair, and maintenance of chromatin structure. As suggested by their functions, misexpression of HP1 proteins is observed in a variety of diseases and has been associated with disease progression and prognosis. HP1 proteins are characterized by both a chromo domain and a chromo shadow domain. They have the ability to dimerize via their chromo shadow domain, and some HP1 proteins form homodimers as well as heterodimers. *Drosophila melanogaster* has two somatic HP1 paralogs other than HP1B, HP1a and HP1C. While HP1a localizes mainly to heterochromatin and HP1C localizes mainly to euchromatin, HP1B localizes to both heterochromatin and euchromation. Chromatin-immunoprecipitation analyses of HP1B in HP1a mutants were carried out to study the relationship between HP1B and HP1a. In these HP1a mutants, HP1B is no longer detected. Two models might explain this finding: (1) HP1B is absent in the HP1a mutant, either it is not produced or unstable; or (2) HP1B protein is produced but either does not localize to chromatin or does not stably interact with chromatin. *HP1b* mRNA has been detected in HP1a mutants, and HP1B protein has be detected by Western blot in HP1a mutants. These findings are inconsistent with model 1, and experiments to test model 2 as well as the interactions between HP1a, HP1B, and HP1C are ongoing. .

859C

Repeat-induced Chromatin Silencing in Fruit Flies (*Drosophila melanogaster*). Michael Lee[1], Tingting Gu[2], April Bauer[1], Sarah Elgin[1]. 1) Department of Biology, Washington University in St. Louis, St. Louis, MO; 2) Nanjing Agricultural University, Nanjing, China.

Heterochromatin is a state of epigenetic DNA packaging associated with gene silencing generally observed in repeat-rich regions of the genome. To investigate heterochromatin formation, 256 copies of a 36-bp *lacO* DNA sequence have been inserted into *Drosophila melanogaster* euchromatin using the 1198 landing pad site within the *nesd* gene on the second chromosome, upstream of the expression reporter *hsp-70-white* (*white* expression is required for a red eye phenotype). In control flies with only the *hsp-70-white* reporter at this site (no added repeats) fully red eyes are observed. However, when*lacO* repeats are present upstream of the reporter, silencing of the adjacent reporter gene is observed, reminiscent of position effect variegation (PEV) in which genes can be inactivated through abnormal juxtaposition with heterochromatin. Using quantitative eye pigment assays and eye imaging, we examined the impact of different mutational backgrounds on reporter gene expression. HP1a binding the *lacO*repeats repressed reporter expression, while HP1a depletion induced it. Surprisingly, mutations in any of the H3K9 histone methyltransferases had no effect. ChIP assays were used to look for proteins that bound around the *lacO* repeats. High levels of Heterochromatin Protein 1a (HP1a) were found compared to relatively low levels for modified histone H3K9me2, confirming the genetic analysis and suggesting an alternative method of heterochromatin silencing induced by the *lacO* repeats. Mutants for the siRNA and piRNA pathways also had no impact, and a bioinformatics analysis revealed no exact piRNA matches from the *lacO* repeats to the Drosophila genome. However, piRNA mutant *maelstrom* and mutants for histone deacetylase (HDAC) complex subunit Sin3a resulted in a significant increase in reporter expression. Considering that the *maelstrom* product has a HDAC interaction domain, it is possible that repeat induced silencing works via histone deacetylation rather than histone methylation. Future work will focus on the impact of HDACs on heterochromatin formation induced by *lacO* repeats. .

860A

Comparative analysis of HP1a, HP1B, and HP1C functions in *Drosophila melanogaster*. Nicole Riddle. Biology, University of Alabama at Birmingham, Birmingham, AL.

HP1 (heterochromatin protein 1) proteins belong to a highly conserved protein family, occurring in all major eukaryotic lineages. HP1 proteins are a class of non-histone chromosomal proteins and were named based on the prominent heterochromatin association of the founding member, HP1a from Drosophila. The structure of HP1 proteins is distinct, with an N-terminal chromo domain joined to a C-terminal chromo-shadow domain via a variable hinge domain. Despite the shared structure, functions of HP1 proteins are highly variable and include roles in chromatin structure, gene regulation, and DNA repair. HP1a, HP1B, and HP1C are the three somatically expressed HP1 homologs in *Drosophila melanogaster*. As part of a comparative study of HP1 proteins, we have generated null mutants in*HP1b*. While mutants lacking HP1a are lethal at the third instar stage and mutants lacking HP1C only rarely survive to adulthood, we find that *HP1b* mutants are mostly homozygous viable. *HP1b* mutants show decreased fertility, some decreased survival, and delayed development. HP1B is an enhancer of PEV, and its transcription start site localization suggests that HP1B is a transcriptional activator. It is mostly associated with transcriptionally active genes and found in an acetylation-rich, open chromatin environment. Curiously, HP1B is preferably localized to genes with a long first intron and might play specialized role in their regulation. Comparing the HP1B localization pattern to those of HP1a and HP1C, we find that HP1B shares the majority of its binding sites with HP1C, while all three HP1 paralogs are found together at approximately 500 genes. We are currently exploring the genetic interactions between HP1a, HP1B, and HP1C using double-mutant analysis. We find that *HP1b/HP1c* double-mutants are viable to the pupal stage, with a very small number

of flies emerging. Overall, our results indicate that the interactions between HP1 family members are complex and suggest that the presence of multiple HP1 proteins will have to be taken into account when examining their various functions. .

861B

Heterochromatin spreading is a common signature of exposure to metals and organic compounds. Katherine Silkaitis[1], Cristina Valente[1,2], Alan Branco[1], Bernardo Lemos[1]. 1) Environmental Health, MIPS, Harvard School of Public Health, Boston, MA; 2) Faculdade de Medicina, University of São Paulo, Brazil.

Metals and organic compounds, such as pesticides and plasticizers, can exert toxicity through perturbations in epigenetic pathways. We investigate the ability of 24 metals and organic compounds to alter chromatin states in a Drosophila model of position-effect variegation. We determined that exposure to cadmium chloride, lithium chloride, and 2,4,5-trichlorophenoxyacetic acid caused expansion of heterochromatin and significantly silenced the *white* gene at the *w[m4h]* locus. Zinc acetate and vinclozolin were milder enhancers of variegation. To investigate the locus-specific effects of the heterochromatin spreading, we utilized *white* variegating strains with the gene translocated to other euchromatin-heterochromatin boundaries. We identified *white* variegating genotypes whose phenotypes are not enhanced upon toxin exposure. We thus conclude that metal and organics-induced changes to chromatin state may be specific to certain variegating w+ insertion sites and translocations. .

862C

Using FCS methods to study chromatin compaction and the dynamics of heterochromatin formation. Amy Strom[1,2], Gary Karpen[1,2]. 1) UC Berkeley, Berkeley, CA; 2) Lawrence Berkeley National Laboratory, Berkeley, CA.

Compartmentalization is a theme used throughout all kingdoms of biology to create functionally distinct units within a complex cellular environment. Even within membrane-bound organelles, subcompartments can exist that are not membrane bound, yet are still physically distinct from surrounding space. The eukaryotic nucleus has a large variety of nuclear bodies that fall within this category: Nucleoli, PML bodies, heterochromatin, to name a few. Some of these bodies self-assemble onto specific DNA sequences, while others seem to be completely sequence-independent. Heterochromatin is unique in that it is assembled onto a variety of sequence classes from multiple chromosomes that associate to create the heterochromatin domain. The process of nuclear body assembly is often studied biochemically, but not often biophysically. Fluorescence Correlation Spectroscopy methods like Number and Brightness and Raster Image Correlation Spectroscopy allow new insights into the biophysical study of diffusion, complex formation and chromatin compaction during the process of heterochromatin formation.

863A

Structure/Function dissection of the *Fab-7* boundary within the context of the bithorax complex: the role of the GAGA factor. Fabienne Cléard, **François Karch**. Dept Genetic and Evolution, Univ Geneva, Geneva, Switzerland.

Bithorax complex regulation along the AP axis results from the sequential "opening" of segment-specific regulatory domains along the chromatin fiber of the BX-C. This so called "open for business" model functions in part thanks to the existence of boundary elements that demarcate on the chromatin the extends of opening of each regulatory domain in a given segments. For instance, the *Fab-7* boundary ensures that upon opening of *iab-6* in A6, open status does not invade the neighboring *iab-7* domain. In the case of a *Fab-7* boundary deletion, active state of *iab-6* spreads in *iab-7* leading to its ectopic activation one segment too anteriorly, and hence causing the homeotic transformation of A6 into A7. In A6, *Fab-7* also protects the active *iab-6* domain from being silenced by the inactive *iab-7* domain. At the chromatin level, *Fab-7* is composed of 3 major and constitutive DNAse hyper-sensitive sites, HS1, HS2 and HS3. A large collection of deletions induced by imprecise excision of a P-element transposon as well as analysis in ectopic context, enabled us and the Schedl's lab in Princeton to determine that the boundary region lies within HS1 and HS2, and that HS3 corresponds to a Polycomb-Response-Element (PRE) belonging to *iab-7*. Unlike all other boundary of the BX-C, *Fab-7* does not harbor any binding sites for the CTCF factor. *Fab-7* share however with *Mcp* and *Fab-8* the presence of binding sites for the GAGA factor.
To further characterize *Fab-7* we used gene conversion to create a phiC31-based integration platform that enables us to mutagenize at will the region spanning HS1-HS3. With this tool we rapidly determined that the sequences critical to the boundary lie solely within HS1 (350bp-long). We also find that a sequence corresponding to HS1 of *D. pseudobsura* sharing only 26% homology with the*melanogaster* sequence rescues the *Fab-7* boundary phenotype. 6 GAGA binding motifs are found within HS1, though at different locations between the *melanogaster* and *pseudobscura* elements. Mutating 6 of these GAGA binding sites within the *melanogaster* HS1 sequence leads to a *Fab-7* phenotype similar to the deletion of the whole HS1.

864B

The Bithorax *Fab-7* insulator element: A paradigm for developmental modulation of insulator function. Daniel Wolle[1], Fabienne Cleard[2], Tsutomu Aoki[1], Girish Deshpande[1], Paul Schedl[1], François Karch[2]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Department of Genetics and Evolution, University of Geneva, 30 quai E. Ansermet, 1211, Geneva-4, Switzerland.

Insulators are *cis*-regulatory elements that play an important role in the 3-dimensional organization of the eukaryotic chromatin. Frontabdominal-7 (*Fab-7)* is a *Drosophila* insulator element that regulates the spatial and temporal expression of the homeotic selector gene *Abd-B* within the Bithorax Complex (BX-C). *Fab-7* is a unique insulator element, whose constitutive activity is generated by *cis*-regulatory sub-elements and trans-acting factors that function in a developmentally restricted manner. Here, we define multiple, minimal sub-elements within *Fab-7*, which display a late stage developmental activity profile. We have identified ~700KDa novel, protein complex (LBC) that selectively binds to these sequence elements in a late stage specific manner. Within each of the three

unusually extended 65bp long LBC elements, the only recognizable motif is GAGAG. Intriguingly, despite being maternally deposited and ubiquitously expressed through out development, GAGA factor (GAF) is an important constituent of this developmentally restricted insulator complex. We are currently investigating if this DNA protein complex is necessary and sufficient for conferring the late-stage specific insulator function. Furthermore, using a comparative evolutionary approach, we demonstrate that the binding sequences for the LBC are considerably diverged across the four different *Drosophila* species; the corresponding insulator proteins, however, are functionally conserved.

865C

Deposition of the histone H3K27me3 mark during fly embryogenesis. Sarah Bowman[1,2], Aimee Deaton[1,2], Heber Domingues[3], Robert Kingston[1,2], Welcome Bender[3]. 1) Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept. of Genetics, Harvard Medical School, Boston, MA; 3) Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

The covalent histone modification H3K27me3 is deposited by gene products of the Polycomb Group (PcG), and is required for gene repression during development. We examined early embryos to discover the how the H3K27me3 pattern arises. H3K27me3 is initially targeted to Polycomb Response Elements at the early blastoderm stage, and it quickly spreads to adjacent sequences during gastrulation. H3K27 methylation is catalyzed by Polycomb Repressive Complex 2 (PRC2). This complex includes the product of extra sex combs (ESC), which protein has a K27me3 binding pocket and stimulates PRC2 activity in vitro. In the absence of ESC, some targets fail to acquire H3K27me3 entirely. At other targets, the K27me3 pattern seen during late embryogenesis resembles that of early embryogenesis. The Polycomb Repressive Complex 1 includes the POLYCOMB protein, which also has a K27me3 binding domain. Embryos mutant for Polycomb show near normal paterns of H3K27me3, although Polycomb is required for repression of many genes. These results provide in vivo evidence for the hypotheses that ESC is required for spreading of the histone mark, and that POLYCOMB is needed for subsequent structural changes to the chromatin fiber.

866A

Sex combs on midleg (SCM) is a subunit of both PRC1 and PRC2, and is required for H3K27 trimethylation. Hyuckjoon Kang[1,2], Kyle McElory[1,2,3], Youngsook Jung[2,4], Artyom Alekseyenko[1,2], Peter Park[2,4,5], Mitzi Kuroda[1,2]. 1) Genetics and Medicine, Harvard Medical School, Boston, MA; 2) Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 3) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA; 4) Center for Biomedical Information, Harvard Medical School, Boston, MA; 5) Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, MA.

The Polycomb group (PcG) proteins are key conserved regulators of development, initially discovered in *Drosophila* and now strongly implicated in human disease. Nevertheless, even in *Drosophila* it remains unclear how PcG complexes (PRC1 and PRC2) are recruited to their target sites and how they function in transcriptional silencing. We hypothesized that analyzing complexes without their initial release from chromatin might reveal previously unknown interactions that are critical to their function. Therefore, we purified PRC1 and PRC2 using BioTAP-XL, a crosslinking and tandem affinity purification approach, followed by separate DNA (ChIP-seq) and protein (mass spectrometry) analysis from the same chromatin-based pulldown. BioTAP-XL mass spectrometry revealed robust recovery of PRC1 subunits, using PC as bait, and strong enrichment of PRC2 components, using E**(Z)** as bait. Furthermore, we identified a select group of new candidate interactors. Interestingly, SCM, previously known as a substoichiometric component of PRC1, was the only PcG protein to strongly co-purify with both PRC1 and PRC2. We confirmed direct binding between SCM and PRC2 using recombinant protein expression. Furthermore, we found that RNAi knockdown of *Scm* resulted in a strong decrease of H3K27me3 on polytene chromosomes, linking Scm to PRC2 function. In contrast, H3K27me3 levels were not significantly diminished after *Pc* (PRC1) knockdown. SCM ChIP-seq occupancy correlated strongly with H3K27me3 in S2 cells, and mass spectrometry of SCM-BioTAP-XL pulldowns revealed similar robust recovery of both PRC1 and PRC2. Taken together, our results strongly suggest that SCM is a key mediator connecting PRC1, PRC2, and H3K27me3 in PcG silencing. Hypotheses for how SCM plays this key role will be presented. .

867B

A Positive Role of Polycomb in Transcription Regulation by Modulating H4K20me1 during Normal Development. Xiangdong Lv[1], Zhijun Han[2], Hao Chen[1], Yuanxin Xia[1], Yi Lu[1], Chenyu Pan[1], Lin Fu[1], Hui Han[1], Gang Wei[2], Yun Zhao[1]. 1) State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai, China; 2) Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, SIBS, CAS, Shanghai, China.

Polycomb group (PcG) proteins were originally shown to play a central role in maintaining heritable gene suppression during the normal development of higher eukaryotes. However, recent studies in Drosophila and mammals imply a potential positive role of PcG proteins in gene transcription although the mechanism is largely unknown. Here, for the first time we report Pc-mediated, E(z)/H3K27me3-dependent positive transcription regulation of *Senseless* (*Sens*), a key transcription factor involved in body plan during normal development. Further mechanistic studies showed that Pc regulates *Sens*expression by regulating H4K20me1 at Sens locus. More importantly, a set of highly conserved genes involved in the developmental process is also positively regulated by Pc with similar mechanism. In summary, we report a novel and general transcription regulation mechanism, that is, Pc-mediated, E(z)/H3K27me3-dependent positive transcription regulation by modulating H4K20me1 during development.

868C

Physical and functional interaction between PIWI and PC is required for transcriptional silencing in *Drosophila*. Tanmoy Mondal[1*], Utpal Bhadra[2], Sncvl Pushpavalli[2], Manika Pal Bhadra[1]. 1) Centre for Chemical Biology, Indian Institute of Chemical Technology, Uppal Road, Hyderabad-500007, India; 2) Functional Genomics and Gene silencing Group, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500007, India.

In fly, *piwi* (P-element induced wimpy testis) encodes a nuclear protein of the Argonaute family that is required for alteration of chromatin structure and gene expression. It functions as an effective modulator for transcription by targeting localization of Pc proteins of the somatic cells in early embryogenesis. We demonstrate a direct and functional interaction between PIWI and Pc in control of transcriptional silencing. Nearly 20-25% PIWI are associated physically with 30% of PC proteins through chromodomain 1 (24-82 AA) in a RNA independent manner. Colocalization of Pc and PIWI at the intense binding sites suggest that PIWI is involved directly in targeting Pc recruitment during early establishment. It is achieved by recruiting two functionally interacting proteins, Polycomb and Piwi and enrichment of histone H3K27 and H3K9 methylation. Loss of function PIWI protein abrogates the accumulation of interacting partner Pc. Therefore, Piwi-PC complex might participate in controlling development regulators and homeotic gene expression and maintenance of cell fate by targeting PcG proteins..

869A

Combgap is a DNA binding protein required for Polycomb group silencing. P Ray, S De, J Kassis. NICHD/NIH, Bethesda, MD.

Polycomb group proteins (PcG) mediate the epigenetic repression of genes involved in development, cellular proliferation and cellular differentiation. PcG proteins form multi-protein complexes that interact with chromatin via DNA elements known as Polycomb group Response Elements (PREs). PREs are made up of binding sites for multiple DNA-binding proteins, including Pho, GAGA Factor (GAF), and Spps. As a first step in understanding the recruitment of PcG protein complexes to DNA, we aimed to identify all of the DNA-binding proteins important for the function of a single PRE from the Drosophila *engrailed* gene, a well-established PcG target. Previous studies from our group showed that a 139 bp region within the *engrailed* PRE contains binding sites for the DNA-binding proteins Pho, Spps, GAF and retains the ability to act as a PRE. This fragment also contains binding sites for two unknown proteins and mutation of one of these sites abrogates PRE activity in functional studies. In order to identify the unknown protein, we performed a biotin-streptavidin pull-down coupled with Mass Spectrometry (MS) using (in parallel) oligonucleotides containing the wild-type and mutated binding site for the unknown protein. MS analysis identified Combgap as one of the candidate proteins. Combgap contains eleven zinc fingers and has been reported to repress the expression of a PcG target gene, *cubitus interuptus*, but the mechanism of this repression was unknown. We observed a colocalization of Combgap with the PRE-binding protein Spps on polytene chromosomes, which suggests an interaction of Combgap with the PcG machinery. A genome wide ChIP-seq study showed Combgap binding at many PREs within the Drosophila genome. Motif analysis identified the sequence GTGT as the likely Combgap binding site, a sequence previously found to be associated with PREs (Schuettengruber et al, 2009). In vitro transcribed and translated Combgap zinc fingers bound to GTGT motifs within the *vestigial* (*vg*) and *engrailed* PREs. Further, the *vg* GTGT motif had previously been shown to be important for pairing sensitive silencing (Okulski et al., 2011). Co-immunoprecipitation experiments show an interaction between the PcG protein Polyhomeotic (Ph) and Combgap. We propose that Combgap acts with other DNA-binding proteins to recruit PcG complexes, in part via an interaction with Ph.

870B

Role of epigenetic factors in aging. Dasari Vasanthi. CCMB, Hyderabad, India.

Individuals within populations show variability in life span and other aspects of age related performances based on several factors like nutrition, stress, environment, etc. The natural diversity in aging is a hallmark of organismal variation. To understand aging process in more detail we started to look for the role of epigenetic factors in this process. Several studies indicate that PcG and trxG are involved in maintenance of expressed state of several developmentally important genes. In one way aging can be interpreted as loosing of such maintenance of the transcriptional status. Preliminary results suggest that some of the PcG and trxG members accelerate or retard aging process in *Drosophila melanogaster*. These findings indicate that some of the PcG and trxG factors modulate organismal longevity.

871C

Baseline X chromosome dosage compensation in heads. Zhen-Xia Chen, Brian Oliver. NIDDK, National Institute of Health, Bethesda, MD.

X chromosome dosage compensation is required for male viability and is achieved by the action of a well-studied complex of proteins and non-coding RNAs. It is assumed that dosage compensation is 2-fold, but the baseline value for dosage compensation, which takes events such as gene regulation into account, is unknown. By using a series of deficiencies on the X and autosomes, along with mutations in the sex determination gene *tra2* to minimize confounding sex-biased expression, we have carefully measured the X chromosome response to gene dose by RNASeq. We observe similar modest dosage compensation for both X chromosome and autosome genes. These data suggest that the X chromosome is neither inherently more robust nor sensitive to dosage change. .

872A

The 1.688 satellite is required for targeting of POF to a transgenic recruitment element. M. Kim, J. Larsson. Umeå University, Umeå, Sweden.

The protein POF (Painting of fourth) is one of the two chromosome-specific systems described in *Drosophila*. It binds expressed genes on the *D. melanogaster* 4^{th} chromosome and maintains their proper transcription in tough heterochromatic environment. In addition, POF targets two loci on the X-chromosome close to the *roX* genes, encoding the two long non-coding RNAs known to be parts of another chromosome-specific system, the MSL-complex. The targeting of POF to these loci, namely *PoX1* and *PoX2,* is *roX* expression-dependent even though it is observed only in females. We have previously identified a high-affinity region which recruits POF ectopically. To determine the factors necessary for this recruitment we constructed a series of transgenes and examined POF binding in salivary glands. Here we show that a putative POF binding site consists of a transcribed gene and several copies of the 1.688 satellite. This satellite is highly overrepresented on the X-chromosome and recruits neither POF nor MSL alone when presented as part of an autosomal transgene. Direction of the adjacent gene transcription but not its chromosomal origin seems to be important for POF binding.

873B

How does the fly identify X chromatin during dosage compensation? Victoria H Meller, Debashish U Menon. Biological Sciences, Wayne State University, Detroit, MI.

Highly differentiated sex chromosomes create a lethal imbalance in gene expression in one sex. To accommodate hemizygosity of the X chromosome in male fruit flies, expression of X-linked genes increases two-fold. The Male Specific Lethal (MSL) complex binds within transcribed X-linked genes and modifies chromatin to increase expression. Mutations in the *roX* RNAs disrupt X localization of this complex lower the expression of X-linked genes and reduce male survival. The mechanism that restricts the MSL complex to X chromatin is not understood. We recently reported that the siRNA pathway contributes to localization of the MSL complex, raising questions about the source of the siRNAs involved. The X-linked 1.688 g/cm^3 satellite related repeats (1.688^X repeats) are restricted to the X chromosome and produce small RNA, making them attractive candidates. We tested RNA from these repeats for a role in dosage compensation. Ectopic expression of single stranded RNAs from 1.688^X repeats enhances the male-lethality of *roX* mutants with defective X recognition. In contrast, expression of double stranded RNA from one 1.688^X repeat dramatically increased male survival and largely restored X-localization of the MSL complex. The striking distribution of 1.688^X repeats, which are near-exclusive to the X chromosome, suggests that they are *cis*-acting elements that identify X chromatin.

874C

New data suggests an ancient mechanism for a chromosome-specific factor. Anna Zeidman, Leila Rieder, Erica Larschan. Brown University, Providence, RI.

In both humans and flies, the gradual evolutionary degeneration of the male sex chromosome (Y) causes a need to equalize gene expression between a single X in males and two X-chromosomes in females. This has led to the evolution of dosage compensation mechanisms. CLAMP (Chromatin-Linked Adapter for MSL Proteins) specifically binds to the X-chromosome in males to recruit the protein complex MSL (Male- Specific Lethal). MSL mediates a 2-fold increase in gene expression specifically on the single male X-chromosome. A second chromosome- specific factor, POF (Painting Of Fourth), specifically binds to the fourth chromosome. The 4th chromosome is an ancestrally derived X- chromosome, supporting the hypothesis that POF's chromosome- specific targeting originates from a dosage compensatory system. To investigate whether POF binding to the X-chromosome is dependent on CLAMP, we performed immunostaining and chromatin immunoprecipitation-qPCR (ChIP-qPCR). Our results suggest that, in males only, MSL complex likely competes with POF, leading to higher POF binding to X in females at the POF-on-X (POF) sites. ChIP-qPCR suggests that POF binding to X may be dependent on CLAMP in males.

875A

The role of chromatin organization in regulating histone gene repeats. K. Boltz, R. Duronio. Lineberger Comprehensive Cancer Center, Univ of North Carolina, Chapel Hill, NC.

During S-phase, millions of histones must be produced to package newly replicated DNA into chromatin. Too few histones will stop replication, and excess histones can cause genome instability. Organisms have thus evolved multiple levels of regulation to maintain proper histone abundance. The canonical histone genes are intronless and are found in clusters. Histone mRNAs are not polyadenylated but instead end in a unique 3' stem-loop, which is important for transcript stability, nuclear export, and translation. A nuclear structure, the histone locus body (HLB), associates with histone gene clusters and contains factors required for histone gene expression and mRNA 3' end processing. Although progress has been made to identify HLB proteins and mechanisms of mRNA processing, little attention has been given to the role chromatin organization plays in regulation of the canonical histone genes. In *Drosophila,* histones are encoded by a tandem array of ~100 copies of a 5kb repeat containing each of the 5 replication-dependent histone genes. It is unknown how many of these repeats are active in any one cell or tissue or if there is differential regulation of repeats across development. To understand how chromatin impacts histone gene expression, we are characterizing cis and trans regulators of the chromatin state at the *Drosophila* histone locus. Mxc is a putative scaffolding protein that organizes the HLB and is required for histone gene expression and pre-mRNA processing. We hypothesize that Mxc also regulates the chromatin state of the histone locus. ChIP-seq is being used to identify Mxc localization across the genome, and we are using FAIRE-seq to compare open chromatin in wild type and *mxc* mutant embryos to determine if Mxc impacts the chromatin state at the histone locus. Finally, to examine regulatory elements

within the histone cluster, we are using a recently developed transgenic histone replacement platform. Prior research showed that the *H3-H4* bidirectional promoter is required for HLB assembly and expression of all four core histones. We have generated transgenic flies with deletions within the *H3-H4* promoter to further delineate the critical sequences for HLB formation and histone gene expression.

876B

The role of PIWI-interacting RNA pathway genes in cell survival after telomere loss. Ana C. Gonzalez, **Rebeccah L. Kurzhals**. Southeast Missouri State University, Cape Girardeau, MO 63701.

Telomeres are specialized structures that protect chromosome ends from being recognized as double-strand breaks. Using the *Bar* and Telomere Loss (BARTL) assay, we can induce loss of a single telomere during eye development, which mimics the situation encountered by a single uncapped telomere. Loss of a single telomere activates the DNA damage response (DDR), which triggers *p53* and *chk2*dependent apoptosis. However, not every cell that has lost a telomere undergoes cell death; a small percentage of cells with abnormal karyotypes persists and continue to divide, which is analogous to cancerous cells. Preliminary data suggests that one mechanism by which cells survive is due to *de novo* telomere addition. We wanted to study factors that could play a role in *de novo* telomere addition. Mutations in *aub*and *armi*, genes involved in the piRNA (PIWI-interacting RNA) pathway, result in telomere fusions and disruption of loading of HOAP, a telomere specific protein, we therefore tested if misexpression of*aub* and *armi* suppressed cell death after telomere loss. In addition, we also screened available misexpression and RNAi lines of genes involved in the piRNA pathway with the hope of better understanding how it works and whether or not the genes tested had an effect on cell survival after induction telomere loss. Our results demonstrate that disrupting the piRNA pathway, in somatic cells, affects cell survival after telomere loss. Surprisingly, both misexpression and RNAi knockdown of *aub* and *armi*, resulted in suppression of cell death after telomere loss. Misexpression of *rhi* suppressed cell death and RNAi knockdown of *rhi* enhanced cell death after telomere loss. Knockdown of *cuff*, *zuc,* and misexpression of *vig* also suppressed cell death after telomere loss compared to controls.

877C

Characterization of *His2Av* in cell survival after telomere loss. Lakshmi P. D. Pulavarthi, Rebeccah L. Kurzhals. Southeast Missouri State University, Cape Girardeau, MO.

Telomeres are nucleoprotein complexes that protect DNA from being recognized as double strand breaks. Telomere loss triggers the DNA damage response pathway and cells are subjected to apoptosis through *chk2* and *chk1* controlled *p53*-dependent apoptotic pathway. However this is not the case for every cell, as some cells escape apoptosis and continue to divide. We are interested in finding out how cells survive after telomere loss. We induced telomere loss using the *Bar* and Telomere Loss (BARTL) assay, this allows us to generate a single broken chromosome end during development. We evaluated the role of *His2Av*, a gene that has a role in epigenetic silencing and DNA repair, for its role in cell survival after telomere loss. Preliminary data demonstrated that flies heterozygous for a mutation in *His2Av* increases cell survival after telomere loss. We evaluated multiple alleles of *His2Av* including a GAL4 induced RNAi knock-down, a deletion of *His2Av*, a hypomorph allele of *His2Av*, and mis-expression line of *His2Av*. We found an increase in cell survival in flies hemizygous for a deletion of *His2Av.* No significant increase in cell survival was observed in *His2Av*hypomorph or for the mis-expression line, and RNAi knock-down of *His2Av* causes cell death even in the absence of DNA damage. We also evaluated the role of *domino*, which loads His2Av on to DNA. Mutations in *domino* increase cell survival after telomere loss indicating that reduced *His2Av* increases cell survival. By which mechanism reduction in *His2Av* increases cell survival i.e either by chromatin silencing or by DNA repair is being investigated. In order to find out the mechanism, we are examining other genes involved in chromatin assembly that also interact with *His2Av*.

878A

Developmental stability and the maintenance of chromatin structure. Frédérique Peronnet[1], Delphine Cuménal[1], Camille Dupont[1], Valérie Ribeiro[1], Vincent Debat[2], Neel B. Randsholt[1]. 1) UMR7622 - Developmental Biology, Institute of Biology Paris Seine, CNRS - UPMC, Paris, France; 2) UMR7205 - OSEB, Systematic and Evolution, MNHN - CNRS, Paris France.

Developmental stability, the buffering of developmental noise, is of paramount importance to maintain symmetry in bilaterians. Indeed, noisiness of growth can cause noisiness in organ size, leading to imperfect symmetry. Developmental noise can be estimated by fluctuating asymmetry that describes the random deviation from perfect symmetry within a population. Overexpression of *Cyclin G* induces an unprecedented, up to 40-fold increase of fluctuating asymmetry, suggesting that *Cyclin G* is a hub in a gene network underlying developmental stability, and making *Cyclin G* a unique tool to address developmental stability. *Cyclin G* genetically interacts with several *Polycomb-group* and *trithorax-group* epigenetic regulators in homeotic gene regulation. Furthermore, Cyclin G binds chromatin, physically interacts with two Enhancers of Polycomb and Trithorax and regulates transcription. Strikingly, *Cyclin G*-induced fluctuating asymmetry increases in mutants for several *Polycomb-group* and*trithorax-group* genes, suggesting that maintenance of chromatin structure might be crucial to buffer developmental noise.

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879B

Cosuppression of *Adh* gene in *Drosophila melanogaster* by insertion of inducible transgene follows typical RNAi pathway. Debabani Roy Chowdhury, Utpal Bhadra. Functional Genomics, Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India.

Post transcriptional gene silencing or PTGS is a cellular mechanism for protecting cells against foreign DNA and active transposons. It operates when multiple copies of the same transgene are introduced in different genomic locations. It reduces the accumulation of homologous RNA with sequence similarity and this is known as cosuppression. Cosuppression, quelling and RNA interference are the different terms of PTGS, which is highly conserved from protozoa to mammals. In *Drosophila*, multiple copies of *Adh* full length transgene, inserted at different locations, showed a gene dosage effect till it reached a threshold which was produced by expression of close to 5 copies (Pal-Bhadra *et al.*, 2002) of the gene. Once the *Adh* transcripts reached the threshold, it triggered rapid degradation of the mRNA. The downstream steps of it exactly followed a similar pattern as found in RNA interference. To determine the triggering components, we used a heat-inducible system for *Adh* mRNA degradation pathway. Two different constructs were used, which were full length transgene and *HSP70* promoter-*Adh* reporter hybrid transgene. In two *Hsp70-Adh* copies, heat induction increased the *Adh* transcripts dramatically as expected; while in 5 copies of *Adh*, a similar induction decreased RNA level instantly. Our studies showed that this cosuppression could easily degrade *Adh* mRNA within 5 minutes of heat treatment. This rapid but complete RNA turn over process could be the spontaneous response of cell surveillance to viral infection and transposon silencing. This led to the strong speculation that these transcripts must form structures *in vivo* mainly by complementary annealing which followed an endonucleolytic cleavage by RNase III. This might act as a homology guide for triggering the RNAi-like degradation process.

880C

Digitor, an Essential Protein with Homology to Mammalian ATMIN is Involved in Brain Development and Oxidative Stress Pathways in Drosophila. Saheli Sengupta, Changfu Yao, Uttama Rath, Jack Girton, Jorgen Johansen, Kristen Johansen. Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA.

Using yeast two-hybrid interaction assays we identified Digitor (CG14962) a zinc-finger protein with six TQT motifs that interacts with Skeletor's CTD. Skeletor localizes to interband regions of polytene chromosomes during interphase and transient expression studies of GFP-tagged Digitor indicate a nuclear localization for Digitor. Furthermore, pulldown assays confirm a physical interaction with Skeletor as well as with cut-up, a dynein light chain protein of 8 kDa. This interaction was also documented *in vivo* in S2 cells where ectopically expressed cut-up-mCherry redistributes completely to the nucleus in presence of high Digitor-GFP expression compared to its ubiquitous distribution in the absence of overexpressed Digitor-GFP. We have generated transgenic flies containing Digitor-GFP under UAS-Gal4 control. Under non-stressed conditions when driven by the sgs3-Gal4 driver, Digitor-GFP not only binds whole polytene chromosomes (global binding) but it also binds, in higher amounts, to specific chromosomal locations (discrete binding) many of which co-stain with active RNA polII, indicating a possible role for Digitor in transcriptional regulation. Digitor-GFP is released from its discrete chromosomal locations immediately after heat shock. Interestingly, the discrete banding pattern is restored after ~5 hours' recovery from heat shock. Multiple sequence alignments indicate that Digitor may be the *Drosophila* homolog of the ATM-kinase interacting protein ATMIN, sharing the zinc-fingers and SQ/TQ motifs that are hallmarks of DNA damage response proteins. In order to initiate functional studies of Digitor we identified a P-element insertion that by RT-PCR analysis appears to be a null allele. Homozygous mutants for Digitor give rise to late pupal lethality with no escapers. Mutant Digitor larvae have smaller brains and exhibit increased DNA breaks as identified by TUNEL compared to the WT. Furthermore, heterozygous mutant flies have increased susceptibility to acute exposure to paraquat suggesting that Digitor is involved in signaling pathways combating acute oxidative stress.

881A

A microRNA minimizes the phenotypic impact of genomic diversity. Justin Cassidy[1], Aashish Jha[2], Diana Posadas[1], Ritika Giri[1], Koen Venken[3,4], Jingran Ji[1], Hongmei Jiang[5], Hugo Bellen[3,4], Kevin White[2], Richard Carthew[1]. 1) Molecular Biosciences, Northwestern University, Chicago, IL; 2) Institute for Genomics and Systems Biology, Departments of Human Genetics and Ecology and Evolution, University of Chicago, Chicago, IL; 3) Department of Molecular and Human Genetics, Howard Hughes Medical Institute, Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; 5) Department of Statistics, Northwestern University, Evanston, IL.

Gene expression has to withstand stochastic, environmental, and genomic perturbations. For example, in the latter case, 0.5%-1% of the human genome is typically variable between any two unrelated individuals. Such diversity might create problematic variability in the activity of gene regulatory networks and, ultimately, in cell behaviors. Using multigenerational selection experiments, we find that for the Drosophila proneural network, the effect of genomic diversity is dampened by miR-9a-mediated regulation of senseless expression. Reducing miR-9a regulation of the Senseless transcription factor frees the genomic landscape to exert greater phenotypic influence. Whole-genome sequencing identified genomic loci that potentially exert such effects. A larger set of sequence variants, including variants within proneural network genes, exhibits these characteristics when miR-9a concentration is reduced. These findings reveal that microRNA-target interactions may be a key mechanism by which the impact of genomic diversity on cell behavior is dampened.

882B

piRNA-mediated repression during female germline development. Pauline Marie, Stéphane Ronsseray, Antoine Boivin. Developmental Biology Laboratory, IBPS, CNRS, Universite Pierre et Marie Curie, Paris, France.

Maintaining the genome integrity is particularly important in the germline which transmits the genetic information over generations.

The activity of transposable elements in animals is regulated by small non-coding RNAs associated with PIWI family proteins (piRNAs). This regulation has extensively been studied during *Drosophila* gametogenesis, but little is known about piRNA-mediated silencing during germline development. Indeed, maintenance of genome integrity over generations requires that regulation takes place throughout the entire life of germ cells. We investigate the functional and molecular properties of the piRNA-mediated repression during *Drosophila* female germline development. Using couple of silencer and target transgenes deriving from the *P*element, we have established the existence of a functional repression during all larval stages as well as in pupae. Despite huge differences between larval Primordial Germ Cells and adult ovariole cells, we show, using TRIPlines (modified miRNA), that most of all the known partners of both the primary and secondary piRNA pathways in the adult germline are also necessary during development. Moreover, we analyze an incomplete silencing discovered in adult ovaries that resembles variegation. We show that this phenomenon takes place during development as well. Is this a developmental epigenetic memory that exists throughout germline development or a fluctuating state is the question that we address.

883C

Subtelomeric regions and transposable element repression in *D. melanogaster*. Laure Teysset, Amna Asif-Laidin, Stephane Ronsseray. Developmental Biology Laboratory, IBPS, CNRS, Universite Pierre et Marie, Paris, France.

The genome of *Drosophila melanogaster* populations has been recently invaded by the *P* element. Then, a mechanism of repression of *P* transposition has been established, which shows an epigenetic transmission over generations. This repression involves at least one copy of the *P* element inserted in Telomeric Associated Sequence (TAS) at the 1A cytological site of the *X* chromosome and is able to repress the activity of up to 80 euchromatic *P* copies in the genome. A transgenic model mimicking *P* repression has been established, where a *P* transgene inserted in TAS can repress a homologous euchromatic *P* transgene in the female germline. Finally we have shown that this repression in *trans* is a general repression mechanism which has been co-opted by a transposable element to regulate its activity after entering in the *drosophila* genome. This mechanism has been called the *trans*-silencing effect (TSE) and involves the PIWI-interacting RNAs (piRNAs) pathway as well as heterochromatin formation. Those piRNAs are implicated in the regulation of transposable elements in gonads. TAS are subtelomeric regions composed of non-coding repeats lying between the terminal retrotransposon array and the euchromatin containing genes and are also known to be a strong producer locus of piRNAs.
Very few is known about the TAS locus, but since it seems to be a master locus for establishment of the *P* element repression and the TSE, we are interesting in characterizing structural and molecular properties of this locus.

884A

Dynamic turnover of transposable elements in the *Drosophila simulans* clade supports an evolutionary arms race with the host silencing machinery. Jeffrey Vedanayagam, Daniel Garrigan. Department of Biology, University of Rochester, Rochester, NY.

Transposable elements (TEs) are selfish genetic elements that are abundant throughout the genomes of most living organisms. A recently identified piwi-interacting RNA (piRNA) pathway has been shown to defend against TEs in the *Drosophila* germline. piRNAs are 23-29 nucleotide RNAs that are generated from TE-rich heterochromatic regions in the genome. In this study, we use the piRNA repertoire as a proxy to characterize TE diversity in three closely related species in the *Drosophila simulans* clade. The crucial role of the host silencing machinery in defending against TEs, and the nature of selfish genetic elements to self-propagate, posits a classic "arms race" scenario. The host-TE conflict predicts both dynamic turnover of TEs and rapid evolution of piRNA pathway genes in the *D. simulans* clade. To measure the rate of TE turnover, we sequence 0-2 hour maternally derived piRNAs and estimate the diversity of abundant, rare and lineage-specific TEs. A hierarchical clustering analysis of comparative piRNA expression shows little evidence for sharing between species and an abundance of lineage-specific TEs, indicating a rapid turnover of TEs within species. Furthermore, McDonald-Kreitman tests on whole-genome polymorphism and divergence data identify 14 genes in the piRNA pathway as having experienced recent positive selection in *D. simulans* and one gene in *D. mauritiana*. This suggests that TE repertoire/activity of the host genome may play a role in exerting different selective pressures on the piRNA pathway effector proteins. In sum, using the piRNA repertoire as a novel approach to characterize TE diversity, we show dynamic turnover of TEs and rapid evolution of piRNA pathway genes in the *D. simulans* clade, consistent with an "arms race" prediction. Finally, identifying lineage-specific TEs and rapidly evolving piRNA pathway genes also lays the foundation for testing the effect of TE/piRNA dynamics on potential "hybrid dysgenesis"-like phenotypes in inter-specific hybrids from the *D. simulans* clade.

885B

Tagging the iab-8ncRNA of the bithorax complex with mCherry. Yohan El bali, Dragan Gligorov, Robert Maeda, François Karch. Dept of Genetic and Evolution University of Geneva, Switzerland.

Parasegment-specific expression of *Ubx, abd-A* and *Abd-B* is controlled by large *cis*-regulatory domains (*abx/bx*, *bxd/pbx*, *iab-2* through *iab-8*). Each *cis*-regulatory domain is subject to transient transcription that give rise to numerous ncRNA during early embryogenesis. A very large ncRNA (the iab-8ncRNA, 92kb-long) is transcribed from early embryogenesis throughout all larval stages until adulthood. The promoter of this large ncRNA lies just downstream from the *Abd-B* homeobox, within the *iab-8* regulatory domain. As expected from a promoter lying within *iab-8*, the spatial expression of the *iab*-8ncRNA is restricted to the CNS in PS13 and PS14. Transcription proceeds towards *abd-A* throughout the entire *iab-7- iab-3* regulatory domains to reach the *abd-A* promoter. Remarkably, the*iab-8ncRNA* is spliced out, picking up an exon in each of the *iab* regulatory domains. The splicing pattern appears conserved in the 13 *Drosophila* species that have been sequenced. Curiously, the intron-exon junctions are the most conserved as if it was the act of spicing rather than the sequence of the exons that matters. The *iab-8ncRNA* is the template of a microRNA (miR-iab-8)

whose sequence lies within *iab-3*. A failure to produce miR-iab-8 leads to both male and female sterility. In agreement with the iab-8ncRNA spatial expression that is restricted to the CNS in PS13 and 14, the sterility phenotype appears to originate from a behavioral problem, rather than a defect in gameto-genesis. To better follow expression of the iab-8ncRNA during larval and pupal stages we have inserted an open-reading frame encoding mCheryy into exon 3 (using our *iab-6* integration platform). Unexpectedly, we fail to detect mCherry in the CNS at any stage of development. This problem does not stem from a mistake into the construction of our targeted mChery, because we do observe strong fluorescence into the secondary cells of the male accessory gland. Male-specific expression originates from an alternative promoter within *iab-6* that was discovered in the large RNAseq dataset published in 2007. Absence of mCherry expression within the CNS does not result neither from exon 3 exclusion resulting from the insertion of the mCherry ORF. .

886C

Lobe-less RNA is a new member of Polycomb group genes and essential for mushroom body morphogenesis in *Drosophila*. Sachi Inagaki[1], Masanao Sato[3,4], Natsuki Nakamura[2], Satoru Kobayashi[3,4], Yuji Kageyama[1,2]. 1) Research Center for Environmental Genomics ,Kobe University, Kobe, Japan; 2) Department of Biology, Graduate School of Science, Kobe University, Kobe, Japan; 3) Okazaki Institute for Integrative Bioscience, Okazaki, Japan; 4) National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan.

It has been shown that long noncoding RNAs (ncRNAs) are involved in a variety of biological phenomena including epigenetic regulation of homeotic genes expression. However, physiological roles of long ncRNAs in the central nervous system are still largely unknown. In previous studies, we have performed screening for long ncRNAs in *Drosophila* and uncovered dozens of long ncRNA candidates (Inagaki *et al.*, 2005). Here we show that one of the candidates Lobe-less (LOL) RNA is a new member of Polycomb group genes and essential for mushroom body morphogenesis in *Drosophila*. LOL RNA is expressed in neural progenitor cells (neuroblasts) and specifically localized in neuclei. To elucidate the function of long ncRNAs in *Drosophila*, we established a small deletion strain that lacks the whole transcribed region of LOL RNA. We found that the *lol* mutant flies showed malformation of the vertical lobes of the adult and larval mushroom body. DNA microarray analysis of adult head revealed that expressions of more than 2,000 genes were statistically changed in *lol* mutant flies. Since genomic loci of *lol* target genes show obvious tendencies to form gene clusters, we investigated genetic interaction between *lol* and chromatin modifier complex PRC1. Polycomb phenotypes of *Pc-G* mutant males were enhanced by the presence of the *lol* mutations, and gain-of-function alleles of*Antennapedia* showed higher penetrance and severer phenotypes of antenna-to-leg transformation. Taken together, our results indicate that Lobe-less RNA is a new member of *Pc-G* and essential for mushroom body development. Current study provides the first evidence that long noncoding RNA contributes in establishment of integrative neural circuits in the brain.

887A

Role of specialized ribonucleoprotein granules in germline development in Drosophila. Alexey L. Arkov[1], Ming Gao[1], Travis C. Thomson[2], T. Michael Creed[1], Shikui Tu[3], Sudan N. Loganathan[1], Christina A. Jackson[1], Nhan Huynh[1], Jimiao Zheng[1], Patrick McCluskey[1], Yanyan Lin[1], Scott E. Collier[5], Jarred Koerner[1], Zachary Ryne[1], Zhiping Weng[3], Paul Lasko[4], Melanie D. Ohi[5]. 1) Department of Biological Sciences, Murray State University, Murray, KY; 2) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; 3) Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester, MA; 4) Department of Biology, McGill University, Montreal, Canada; 5) Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN.

Germ cells give rise to eggs and sperm and therefore, to the next-generation organisms. These cells contain special ribonucleoprotein organelles referred to as germ granules. Although these granules contain components that have been individually shown to be crucial for germline development and genome integrity of germ cells, the assembly, structure and function of these large organelles are not well understood. Here, using single-particle EM structural approach, we present the first structural snapshot of the principal component of the granules, scaffold Tudor protein. Our study suggests that Tudor is a dynamic molecule which plays a major role in the assembly of germ granule's self-sufficient functional unit that consists of a Piwi family protein, small Piwi-interacting RNAs (piRNAs), ATP-dependent RNA helicase and ATP-generating glycolytic enzymes. Our data provide evidence for the crucial role of this Tudor-mediated germ granule structure in the silencing of transposable elements and germ cell formation and suggest the mechanistic principles of germ granule assembly and function.

888B

Temporal and spatial control of the Smaug RNA binding protein in the early embryo. Wendy Cao[1], Alexander Marsolais[2], Matthew Cheng[2], Najeeb Siddiqui[1], Craig Smibert[1,2], Howard Lipshitz[1]. 1) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Biochemistry, University of Toronto, Toronto, Ontario, Canada.

Maternally loaded mRNAs control early embryo development in many animals. RNA binding proteins and small RNAs regulate the translation, stability and subcellular localization of these maternal mRNAs. In *Drosophila,* the highly conserved RNA binding protein, Smaug (SMG), is synthesized upon egg activation, and is required for translational repression and degradation of a large subset of the maternal transcripts during the maternal-to-zygotic transition (MZT). Both SMG protein and *smg* mRNA are cleared at the end of the MZT although SMG is transported into, and persists in, the pole plasm and pole cells. We have shown that protein instability is directed by the C-terminal portion of SMG whereas RNA instability is directed by elements in *smg*'s 3'UTR. When either the C-terminus of the protein or the 3'UTR elements are deleted, SMG protein persists in the soma beyond blastoderm cellularization. We are mapping both the protein and RNA instability elements; identifying the mechanisms and factors that confer instability; and investigating the biological

functions of SMG clearance from the soma. In addition we are investigating the mechanisms and functions of SMG accumulation in the pole plasm.

889C

Extensive cross-regulation of post-transcriptional regulatory networks in *Drosophila*. Marcus Stoiber[1,2], Sara Olson[3], Gemma May[3], Michael Duff[3], Jan Manent[4], Robert Obar[4], K. G. Guruharsha[4,5], Spyros Artavanis-Tsakonas[4,5], James Brown[2,6], Brenton Graveley[3], Susan Celniker[2]. 1) Department of Biostatistics, UC Berkeley, Berkeley, CA; 2) Department of Genome Dynamics, Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Department of Genetics and Developmental Biology, Institute for Systems Genomics, University of Connecticut Health Center, Farmington, CT; 4) Department of Cell Biology, Harvard Medical School, Boston, MA; 5) Biogen Idec Inc, 14 Cambridge Center, Cambridge, MA 02142 USA; 6) Department of Statistics, University of California Berkeley, Berkeley, CA.

In eukaryotic cells, RNAs exist as ribonucleoprotein particles (RNPs). Despite the importance of these complexes in many biological processes including splicing, polyadenylation, stability, transportation, localization, and translation, their compositions are largely unknown. We affinity purified 20 distinct RNA binding proteins (RBPs) from cultured *Drosophila melanogaster* cells under native conditions and identified both the RNA and protein compositions of these RNP complexes. We identified "high occupancy target" (HOT) RNAs that interact with the majority of the RBPs we surveyed. HOT RNAs encode components of the nonsense-mediated decay and splicing machinery as well as RNA binding and translation initiation proteins. The RNP complexes contain proteins and mRNAs involved in RNA binding and post-transcriptional regulation. Genes with the capacity to produce hundreds of mRNA isoforms, ultra-complex genes, interact extensively with heterogeneous nuclear ribonuclear proteins (hnRNPs). Our data is consistent with a model in which subsets of RNPs include mRNA and protein products from the same gene, indicating the widespread existence of auto-regulatory RNPs. From the simultaneous acquisition and integrative analysis of protein and RNA constituents of RNPs we identify extensive cross-regulatory and hierarchical interactions in post-transcriptional control.

890A

The RNA interactome of early Drosophila embryo. Vasily Sysoev, Bernd Fischer, Christian Frese, Sophia Föhr, Alfredo Castello, Matthias Hentze, Jeroen Krijgsveld. European Molecular Biology Laboratory, Heidelberg, Germany.

Embryos, whose dividing nuclei are generally transcriptionally silent during the first hours post-fertilization, depend on maternally deposited mRNAs for their development during the early stages. During the maternal-to-zygotic transition (MZT), maternal transcripts are degraded and give place to newly transcribed zygotic mRNAs, hence the RNA and protein composition of the embryo changes drastically. Information on the dynamicity of the mRNA-bound proteome is essential for understanding of the molecular mechanisms underlying the MZT and subsequent development. We have adapted the method of interactome capture developed by Castello et al. (Cell, 2012) to identify mRNA binding proteins at the earliest stages of embryogenesis and at stages following the completion of the MZT. The method involves irradiation of embryos with UV light, which cross-links nucleic acids to their direct interaction partners. Isolation of polyadenylated RNAs and stringent washes ensure minimal contamination. To our knowledge, this work represents the first proteome-wide identification of RBPs in a developing multicellular organism. In our experiments we identified hundreds of proteins, many of which have not been reported previously to play a role in RNA metabolism. Our data show that more than a hundred RBPs, both known and novel, are selectively enriched in the RNA-bound fraction at one of the stages. This could be due to post-translational regulation of the RNA binding properties of these proteins or to stage-specific expression of their target mRNAs. Many of these are implicated in early developmental processes, such as formation of the central nervous system and trachea. Based on our findings and available phenotypic data, we are addressing the mechanisms of action of the proteins in these processes. Our data also indicate that seven splicing factors bind RNA in a stage specific manner, and six of these are overrepresented in the RNA-bound fraction of early embryos. This was unexpected as transcription and pre-mRNA processing rates are very low in the rapidly dividing nuclei of embryos at the early stage. We are investigating possible unexplored roles of these splicing factors in early development.

891B

Isoform-specific function of Squid in *bicoid* mRNA localization. Evan Abbaszadeh, Elizabeth Gavis. Department of Molecular Biology, Princeton University, Princeton, NJ.

Transport of mRNAs to distinct cellular locations is a conserved mechanism to spatially restrict protein synthesis. In many cells, several localization pathways may operate concurrently and, despite sharing localization factors or molecular motors, transport RNAs to distinct subcellular domains. The extent to which these different localization pathways are interconnected, and whether RNAs that share common localization steps are transported together, remains poorly understood. To investigate these questions, we have focused on two mRNAs, *bicoid* (*bcd*) and *gurken* (*grk*), which are localized respectively to the anterior and dorso-anterior cortex of the *Drosophila* oocyte. Both RNAs are transported by dynein and accumulate along the anterior cortex. Anterior accumulation of *grk* is transient, however, and*grk* subsequently moves dorsally. Additionally, localized *bcd* and *grk* have both been found to occupy the same electron dense structures, called P-bodies. We have identified a novel role for the P-body component Squid (Sqd), a known regulator of *grk* localization and translation, in the localization of *bcd* RNA. In *sqd* mutant oocytes, *bcd* becomes detached from the anterior cortex during later stages of oogenesis and accumulates along the lateral cortex of the oocyte. The *sqd* gene encodes three protein isoforms, and expression of two of these isoforms partially rescues the dorsolization defect of *sqd*mutants caused by aberrant regulation of *grk*; expression of the third isoform, SqdB, does not. Surprisingly, expression of SqdB in *sqd* mutant oocytes rescues the *bcd* mislocalization phenotype, indicating that different Sqd isoforms have RNA-specific functions. *sqd* has previously been proposed to function in *grk* localization by converting motile dynein carrying *grk* RNA to a static anchor, and work from our lab has shown dynein to be

required for *bcd* anchoring. We are presently using live imaging to investigate whether *sqd* is involved in the dynein mediated maintenance of *bcd* at the anterior of the oocyte.

892C

Independent and coordinate trafficking of single germ plasm mRNAs. Shawn Little[1,2], Kristina Sinsimer[1], Jack Lee[1], Eric Wieschaus[1,2], Elizabeth Gavis[1]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Howard Hughes Medical Institute.

mRNA localization is a conserved mechanism for spatial control of protein synthesis that plays key roles in the generation of cellular and developmental asymmetry. In many cells, different transcripts may be localized to the same subcellular domain, but the extent to which mRNA transport and targeting are coordinated is unclear. We have used quantitative single molecule imaging to analyze the assembly of mRNA-containing granules that populate the germ plasm and are inherited by the nascent germ cells. We find that the germ cell destined transcripts *nanos* (*nos*), *cyclin B* (*cycB*) and *polar granule component* (*pgc*) travel within the oocyte cytoplasm as ribonucleoprotein particles (RNPs) containing single mRNA molecules but co-assemble into multi-copy heterogeneous granules selectively at the posterior pole of the oocyte. The stoichiometry and dynamics of the assembly process suggests that it occurs in a defined stepwise sequence. Granules containing *nos*, *cycB* and *pgc* RNAs are biochemically distinct from granules containing the germline determinant mRNA *oskar* (*osk*) that form concurrently at the posterior pole. Our data suggest that co-packaging of *nos*, *cycB*, and *pgc* mRNAs ensures their effective segregation to future germ cells. Compartmentalization of *osk* into a different granule limits its entry into germ cells, and we show that this exclusion is required for proper germline development.

893A

Location, Location: whole genome study of subcellular RNA localization beyond the early *Drosophila* embryo. Ronit Wilk[1,2], Jack Hu[1], Henry Krause[1,2]. 1) The Terrence Donnelly Centre for Cellular and Bimolecular Research, University of Toronto, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Our group has previously described the use of high resolution FISH to interrogate the prevalence and diversity of subcellular RNA localization during the first 4 hrs of embryogenesis (*Lecuyer et al, 2007*). Surprisingly, of the ~ 3000 transcripts analyzed, approximately 70% were found to be subcellularly localized in dozens of unique distributions. Wishing to see if these numbers extended to other stages of development, whether early localization mechanisms are employed in other tissues, and to see if additional mechanisms exist in the thousands of unique and polarized cell types that are not represented within the early embryo, we have extended these analyses to other stages of development. We have now curated an additional 4,500 genes over the full course of embryogenesis, and approximately 500 in 3rd instar larval tissues (*Wilk et al, 2010*). Although cells in later stage embryos are relatively small, a very high degree of localization was nevertheless observed, raising the minimal rate of subcellular localization during embryogenesis to ~80%. In the much larger cells of the 3rd instar larva, virtually all transcripts observed showed some form of subcellular localization in at least one tissue. The transcripts examined also include a growing number of non-coding RNAs, all of which exhibit some form of subcellular localization. These results, which have been annotated and made available on our 'Fly-FISH' database (*fly-fish.ccbr.utoronto.ca*), provide a rich and unique resource for functional gene analyses.

894B

Decreased expression of SR proteins alters lipid droplet morphology and triglyceride levels in Drosophila larvae. Leelabati Biswas[1], Spencer Ng[1], Bijal Kakrecha[2], Alexis Nagengast[1]. 1) Biochemistry, Widener University, Chester, PA; 2) Biology, Widener University, Chester, PA.

The enlargement of adiposomes is a hallmark of obesity, which is emerging as a national epidemic and the fifth-leading cause of death in the United States. Adiposome size is altered in response to changes in the expression levels of spliceosomal proteins and of splicing related (SR) proteins, which function in spliceosome recruitment. In the present study, we evaluated SR protein expression and key post-translational modifications in varying nutrient conditions in *D. melanogaster*. This work clarifies the relationship between splicing related proteins and lipid storage phenotypes in obesity.

895C

Using a histone replacement system to define co-transcriptional interactions between histone modifications and elongating RNA. Michael P. Meers[1], A. Gregory Matera[2]. 1) Curriculum in Genetics and Molecular Biology; 2) Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC.

Histone post-translational modifications (PTMs), including covalent addition of methyl groups to histone N-terminal tails, are thought to influence gene expression in myriad ways, most prominently by remodeling gene promoters to facilitate or prevent access of the core transcriptional machinery. In contrast, methylation at lysine 36 in the histone H3 N-terminal tail (H3K36me) is enriched in gene bodies and within constitutive exons, suggesting that this particular mark may interface with co-transcriptional RNA processing machinery. Indeed, trimethylation of H3K36 has been shown to suppress spurious intragenic transcription, and to interact with chromatin "reader" proteins that bind to elements of the splicing machinery. To define the requirement for H3K36me in RNA processing and maturation, we developed a genetic system for histone gene replacement in *Drosophila melanogaster* to generate *in vivo* models in which all of the replication-dependent histone genes are replaced with copies that are mutant at H3K36, and therefore cannot be conventionally methylated. We find that an arginine mutation (H3K36R) is lethal in late larval stages, indicating a significant role for H3K36me in development. To further investigate RNA splicing and processing phenotypes, we sequenced poly-A mRNA from whole larvae collected

from animals null for the endogenous histone gene cluster and replaced with either wild-type or H3K36R histone genes, via an Illumina stranded, paired-end 100 bp library preparation. To test the effect of H3K36 methylation upon cryptic transcription initiation, we sequenced short capped nascent transcripts from larval nuclei in the same genotypes. Here we report a range of RNA-related defects in H3K36 mutants that indicate the importance of H3K36me in maintenance and homeostasis of co-transcriptional RNA processing. From this data, we seek to delineate classes of RNAs disproportionately sensitive to chromatin perturbation, and to infer a potential mechanism for co-transcriptional crosstalk between histones and elongating RNAs.

896A

The exon junction complex facilitates pre-mRNA splicing via modulation of transcription elongation. J. Akhtar[1], N. Kreim[1], F. Martini[2], G. Mohana[1], J. Mazur[2], H. Binder[2], **JY Roignant**[1]. 1) Institute of Molecular Biology, Mainz, Germany; 2) Institute for Medicine Biometrie, Epidemiologie and Informatic (IMBEI), Mainz, Germany.

Pre-mRNA splicing results in deposition of the exon junction complex (EJC) upstream of exon-exon boundaries on mature transcripts. The EJC is a ribonucleoprotein complex that controls several post-transcriptional functions including RNA localization, translation and nonsense mediated decay. In addition, we have previously demonstrated an important role for the EJC in pre-mRNA splicing (Roignant and Treisman. 2010, Cell 143, 238-50; Malone et al., 2014, Genes Dev. 28, 1786-99). The mechanism underlying this function involves the initial deposition of the EJC at spliced junctions and the subsequent role of its splicing subunits in facilitating the recruitment of the splicing machinery at adjacent introns. Here we demonstrated that the EJC modulates pre-mRNA splicing via a second mechanism that does not require its splicing subunits but instead relies on the modulation of nucleosome positioning and on the rate of transcription elongation by its core subunit Mago nashi. Importantly, these effects seem, at least in part, independent of its binding to exon junctions. Accordingly, decreasing the speed of Pol II was sufficient to rescue exon skipping and the previously reported eye phenotype associated with the lack of the EJC. This effect appears highly specific and limited to EJC since knock down of other spliceosome components did not increase the speed of RNA Polymerase II. Altogether, our data support a mechanism in which the EJC influences pre-mRNA splicing through the modulation of transcription elongation. These results significantly advance our understanding on the emerging connections between chromatin, transcription and pre-mRNA splicing.

897B

***Tet*, the 5-methylcytosine Oxidase, is Essential in Drosophila. F Wang**, S Minakhina, R Steward. Waksman Institute, Piscataway, NJ.

DNA methylation in Drosophila has been controversial and recent genome-wide bisulfide sequencing did not uncover any methylated cytosine. However, Drosophila has one essential *Tet* gene. Drosophila Tet is homologous to the three vertebrate proteins, 5-methylcytosine (5mC) hydroxylases that catalyze the transition of 5mC to 5hmC, resulting in the elimination of the methyl mark on DNA. TET proteins have well-documented functions in the maintenance of vertebrate stem cells, and function as epigenetic regulators of gene expression. Our whole genome bisulfide sequencing revealed no detectable 5hmc in Drosophila DNA. This result raises the possibility that Tet may also catalyze hydroxymethylation of 5mC in RNA (5hmrc) and our antibody staining experiments suggests that 5hmrc indeed exists in Drosophila. Loss of *Tet* function results in strong ovary and brain abnormalities. The nuclear localization of Tet protein implies that *Tet* may function co-transcriptionally and regulate RNA maturation or processing. Together our results suggest that Tet functions in a process other than DNA demethylation.

898C

The level of nuclear 80S ribosomes increases during cell stress. A. Abdullahi, S. Brogna. Bioscience, University of Birmingham, Birmingham, Birmingham, W. Midlands, United Kingdom.

A hallmark of translation initiation is joining of the small and large ribosomal subunits on the mRNA. Although the 40S and 60S subunits are synthesized and assembled in the nucleolus in eukaryotes, it is believed that there are mechanisms that kept them inactive, preventing 80S assembly and translation in the nucleus. The consensus is that translation occurs only in the cytoplasm. Contrary to this view, we have recently reported that translating 80S ribosomes are also present in the nucleolus and other nuclear sites in *Drosophila* (1). By employing our 80S reporter technique coupled to flow cytometer analysis of *Drosophila cells*, we have observed that nuclear 80S are most apparent during S phase. Furthermore, increased levels of nuclear 80S were observed upon serum starvation and other forms of cellular stress. Our observations suggest a role for nuclear translation during cellular stress.

1: Al-Jubran et al. Visualization of the joining of ribosomal subunits reveals the presence of 80S ribosomes in the nucleus. RNA. 2013.

899A

Translational regulation of the grk mRNA during Drosophila oogenesis. Ramses Rodriguez[1], Jacob Merle[1], Philip Frankino[1], Danielle Hindes[1], Malachi Blundon[1], Maya Mills[1], Matthew Fountain[1,2], Scott Ferguson[1]. 1) Department of Biology, SUNY Fredonia, Fredonia, NY; 2) Department of Chemistry, SUNY Fredonia, Fredonia, NY.

During Drosophila oogenesis, Gurken (Grk) expression is required for proper anterior-posterior and dorsal-ventral polarity determination. The maternal mRNA is only translationally active in certain stages of the oocyte development and this expression is controlled by regulating cap- dependent translation. In *spn-B* mutants, *grk* translation is diminished; this mutant DNA repair enzyme is responsible for activation of the ATR/Chk2 mediated meiotic checkpoint leading to Vasa phosphorylation and subsequent inactivation of cap-dependent translation. During cap-dependent translation, Vasa participates in the pre-initiation complex (PIC) as a helicase to the 5'UTR of an mRNA. In the 5' UTR of certain mRNAs, secondary structures function as an internal ribosomal entry sites (IRES) that can facilitate PIC-independent ribosomal assembly and subsequent translation. In *spn-B* and *Lnk* double mutants, Grk expression is restored

to functional levels. Lnk participates in the insulin/insulin-like peptide signal transduction pathway; mutant lnk interrupts cap-dependent translation by indirect inhibition of downstream Target of Rapamycin (TOR) kinase activity on eIF4E binding protein (4EBP). This ultimately allows it to inhibit eIF4E, which is necessary for PIC assembly. We believe that *lnk* suppresses the diminished Grk translation caused by *spn-B* mutants by promoting IRES-mediated translation of the *grk*mRNA. We have developed *grk* 5'UTR reporter constructs to test our hypothesis that the *grk* mRNA contains an IRES. *In vitro* translation of the *grk* 5' UTR constructs will be performed with ovarian lysate from wild type and rapamycin-fed flies to yield biochemical insights into *grk* translation regulation.

900B

Long noncoding RNA-Proteins interaction module initiates heterochromain. Indira Bag[1], Manika Pal Bhadra[2], Utpal Bhadra[1]. 1) Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500007, INDA; 2) Centre for Chemical Biology, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500007, India.

Majority of long non coding RNA have yet to be characterized throughly. A significant number have been shown to localize subcellular compartments and associated with different human diseases. Rapid cell division required high packaging heterochromatin which is recently known to give rise RNAi based small variety of RNAs. *Drosophila* new RNAi component dRm62 operates nuclear organization, heterochromatin formation. Here, we identify a remarkable and unexpected long ncRNAs generated from 5' regulatory sequences of the 7 copies *mini-w* model as the initial target for dRm62 protein. The dRm62 dependent heterochromatin triggers by long non coding RNA-protein interaction module can recruits different heterochromain markers at the promoter of *mini-w* array, as well as normal heterochromatin of minichromosome. Degradation of ncRNAs by RNases treatment suppresses silencing by eleminating different histone methylations binding at the *mini-w* arrays. *In vivo* binding assay in developing embryos indicate that long noncoding RNA may target regulatory dRm62 proteins directly which interplay accumulation of different heterochromatin markers. Therefore RNA-protein interaction modules play a crucial role in *de novo* heterochromatin formation, accomodating different markers, which are important for chromosome behaviour, evolution and development.

901C

Genome editing in *Drosophila* to reconstruct the evolutionary change in the transcriptional control and processing of RNase P RNA, an essential ribozyme. Geeta Palsule[1], Sathiya Manivannan[1], Lien Lai[2], Venkat Gopalan[1,2], Amanda Simcox[1]. 1) Molecular Genetics, The Ohio State University, Columbus, OH; 2) The Department of Chemistry and Biochemistry, The Ohio State University,Columbus, OH.

Ribonuclease P (RNase P) RNA (RPR) is an essential ribozyme required for the 5' maturation of pre-tRNAs and is the catalytic component of the ribonucleoprotein form of the RNase P enzyme. In all eukaryotes studied so far RPR coding genes are independent and are transcribed by RNA polymerase III (pol III). Whereas in Drosophila RPR is annotated in the intron of a protein coding gene (recipient gene) and lacks elements for RNA pol III transcription. We have found that the intron contains all the sequences necessary for the processing of the RPR but it depends on the recipient gene for expression. Analysis of RPR genes in various animal genomes revealed a striking divide in the animal kingdom that separates insects and crustaceans into a single group in which RPR genes lack signals for independent transcription and is embedded in different protein-coding genes. Our findings provide evidence for a genetic event that occurred approximately 500 million years ago in the arthropod lineage, which switched the control of the transcription of RPR from pol III to pol II. The functional significance of this switch, if any, and how the RPR is processed from the intron remain unknown. Elucidating the processing mechanism might shed light on processing of other RNAs as well. To address this, we are using the CRISPR/cas9 system to reengineer the current pol II-RPR locus in Drosophila to a pol III driven RPR and test the function of this pol III-regulated gene. To examine the role of candidate nucleases in processing the intronic RPR, we plan to carry out an RNAi screen to identify processing factors.

902A

On a wing and a chip - microfluidics for whole organ studies in *Drosophila* development. Cody Narciso[1], Thomas Storey[2], David Hoelzle[2], Jeremiah Zartman[1]. 1) Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN; 2) Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, IN.

Ex vivo culture of the *Drosophila* wing imaginal disc has long provided the *Drosophila* research community with a powerful tool for dissecting regulatory signaling pathways and processes. However, conventional *ex vivo* culture strategies have shown their limitations in regard to the application of exogenous stimuli to a tissue. Devices for application of such stimuli as mechanical forces and electrical gradients are often unwieldy to implement under the constraints of a microscope stage, and running multiple such experiments in parallel is virtually impossible using current methods. The ability to inexpensively fabricate polymeric channels with micron-scale features has led to an explosion in microfluidic research, particularly with biological application. Here, **we present the development of an integrated, multi-chamber, regulated epithelial microenvironment (REM) chip for the culture of Drosophila wing imaginal discs** under real-time observation on a confocal microscope. The chip design **allows for unparalleled simultaneous control over the chemical perfusion rate, in addition to applied mechanical and electrical stimuli.** The REM-Chip enables investigations into underlying connections between a genetic response and the environmental context of the tissue. This will provide a new dimension to our understanding of how tissues grow to a specific size, repair any damage that arises, and maintain homeostasis.

903B

A rationally designed fluorogenic protease reporter visualizes spatiotemporal dynamics of apoptosis *in vivo*. Xiaokun Shu, Tsz-Leung To, Beverly Piggott, Yuh-Nung Jan. Pharmaceutical Chemistry, UCSF, San Francisco, CA.

Apoptosis plays fundamental roles in animal development and disease. However, its visualization in animals has been difficult. We have designed a genetically encoded infrared fluorogenic executioner-caspase reporter (iCasper) that becomes fluorescent upon caspase activation, and does not require exogenous cofactor. iCasper visualizes spatiotemporal dynamics of apoptosis during embryonic development of *Drosophila*, which suggests spatiotemporal coordination between apoptosis and morphogenesis. iCasper also reveals dynamics of apoptosis during tumorigenesis of the larval brain, which may suggest overproliferation-induced apoptosis followed by evasion of apoptosis. iCasper will thus be an important tool in understanding biological function and molecular regulation of apoptosis during development and may shed light on molecular mechanisms of tumorigenesis that require evasion of apoptosis. The designed scaffold can be used to engineer genetically encoded fluorogenic reporters of other proteases, which play important roles in a diverse range of biological processes and disease.

904C

High-throughput analysis of RNAi knockdown efficiency and off-target effects. Hangnoh Lee[1], Michael Buckner[2], Quentin Gilly[2], Haiwang Yang[1], Hina Sultana[1], Cale Whitworth[1], Maria Jaime[1], Satish Kumar[1], Harold Smith[1], Yanhui Hu[2], Stephanie Mohr[2], Norbert Perrimon[2], Brian Oliver[1]. 1) Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA; 2) Drosophila RNAi Screening Center, Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

RNAi-mediated depletion of gene products is a widely used technique, but interpretation of results are often complicated by different degrees of knockdown efficiency as well as off-target effects. To systematically measure both, we generated the largest extant RNAseq profiling dataset of 1,920 RNAi knockdowns of genes encoding 472 transcription factors in S2R+ cells using DRSC reagents. At one day of treatment, we observed reduced expression for 98% of the target mRNAs. Interestingly, we observed a bimodal distribution of knockdown efficiency whose two centers demonstrate 76% and 99% reductions of the target mRNA, respectively. We also assessed possible off-target effects by evaluating 5,105 potential off-target regions from seed-based alignment of 944 RNAi reagents to the reference genome. Among them we found that 2% of the reagents displayed significant reduction of gene expression as off-targets effects ($q < 0.1$), which is less than computationally predicted. Importantly more than a half of those off-target effects were from three reagents (31/61), which contain sequence repeats. Our result shows high efficacy and low rates of off-target effects in *Drosophila* cell-based RNAi. A complete analysis of efficacy and off-target effects genome-wide will aid in the interpretation of RNAi experiments and design of new reagents.

905A

The Transgenic RNAi Project: Reagents, Tools & Validation. Liz Perkins[1], Claire Hu[1], Laura Holderbaum[1], J-Q Ni[2], Shu Kondo[3], Stephanie Mohr[1], Norbert Perrimon[1]. 1) DRSC & TRiP, Harvard Medical School, Boston, MA; 2) TsingHua Univ, China; 3) NIG, Japan.

The TRiP at HMS is entering its last year of NIH funding. Since inception this resource has produced high quality RNAi reagents as well as a robust production pipeline & valuable knowledge on gene functions. Currently. >280,000 TRiP stocks have been distributed by the Bloomington stock center (BDSC). As of 11/14, the collection covers ~9800 unique genes (stocks & in production), including 80% of highly conserved genes. 22% of the stocks encode long dsRNAs that work well in the soma & 78% encode highly efficient short hairpins that are effective in soma & germ line. 70-80% of the TRiP stocks display knock-down efficiencies >50% (see Sopko et al, Dev Cell 2014). Other TRiP reagents include Toolbox stocks, knock-down & overexpression vectors & a vector for the Q-UAS system.
The TRiP has completed production of RNAi sub-libraries of functional gene groups, including kinases, phosphatases & transcription factors. From the BDSC the community will be able to order a specific gene group with 1 click. Also near completion is a collection of RNAi stocks targeting high-confidence fly orthologs of high-confidence human disease-associated genes from the NCBI Online Mendelian Inheritance in Man database. These include congenital, cardiovascular & musculoskeletal diseases, neoplasms & mental disorders. Finally, the TRiP established the RNAi Stock Validation & Phenotypes (RSVP, http://www.flyrnai.org/RSVP.html); a place to find & submit validation & phenotype data on RNAi stocks from the TRiP, VDRC (Vienna) & NIG (Japan). As of 11/14, the RSVP contains 5935 data entries for 4832 TRiP lines representing 2576 fly genes. Also, the RSVP has 27,657 data entries (from FlyBase) for 12,823 RNAi lines representing 10,726 genes. This allows the community to identify & select RNAi reagents with proven efficiencies. Identification of RNAi lines is easily searchable through UP-TORR (http://www.flyrnai.org/up-torr/).

906B

CRISPR/Cas9 mediates efficient conditional mutagenesis in *Drosophila*. Zhaoyu Xue, Menghua Wu, Kejia Wen, Mengda Ren, Li Long, Xuedi Zhang, **Guanjun Gao**. School of Life Sciences, Tsinghua University, Beijing, Beijing, China.

Existing transgenic RNA interference (RNAi) methods greatly facilitate functional genome studies via controlled silencing of targeted mRNA in *Drosophila*. Although the RNAi approach is extremely powerful, it still has lingering concerns of low efficiency and off-targeting. Here, we developed a CRISPR/Cas9-mediated conditional mutagenesis (CMCM) system by combining tissue-specific expression of Cas9 driven by the Gal4/upstream activating site (UAS) system with various ubiquitously expressed gRNA transgenes to effectively inactivate gene expression in a temporally and spatially controlled manner. Furthermore, by including multiple gRNAs in a transgenic vector to target a single gene, we achieved a high degree of gene mutagenesis in specific tissues. Currently, the transgenic gRNA constructs are targeted to the genome by the phiC31-mediated integration approach using a vector carrying an attB sequence to

allow for phiC31-targeted integration at fitted genomic attP landing sites. Therefore, the CMCM system provides a simple and effective tool for gene function analysis, and complements the existing RNAi approach. .

907C

Toward a Complete Set of Germline Null and Modifiable Mutations in *Drosophila*. Graeme Mardon, Ming Fa, Min Li, Rui Chen. GenetiVision Corporation, Houston, TX.

The utility of a model organism largely depends on our ability to manipulate its genome, such as generating mutant alleles and gene tagging. However, this is a major bottleneck for *Drosophila* research. The most flexible and efficient way to modify the genome is to first target a gene with a docking cassette which can then be altered by inserting custom cassettes using recombinase mediated cassette exchange (RMCE). One such system is the Minos mediated integration cassette (MiMIC). However, since Minos integration in the genome is not completely random, a significant fraction of genes in the genome remain untargeted. To address this problem, we designed a CRISPR-Cas9 mediated cassette targeting approach. Specifically, we are using CRISPR-Cas9 to create stocks carrying a MiMIC-like intronic or coding disruption docking cassette in genes that lack nearby transposon insertions. Both types of cassettes will create strong loss-of-function alleles and serve as docking sites for subsequent manipulations of the targeted gene. This resource will have multiple utilities. First, it will fill the gap of existing collections by specifically targeting genes that are resistant to transposable element insertions. Second, the docking cassette at the targeted site will enable rapid and flexible subsequent manipulations of the targeted gene, such as transcript and protein tagging by RMCE and generation of additional mutant alleles. We will maintain all transgenic lines, provide a readily searchable database for researchers to identify stocks of interest, and offer these transgenic flies to scientists at an affordable price.

908A

Anchoring proteins away: a fast and regulated method to deplete proteins in the cell. Pablo Sánchez Bosch, Julia Pepperl, Konrad Basler. University of Zürich, Zürich, Switzerland.

Protein loss-of-function (LOF) analysis allows the study of a certain gene function. Traditionally, it has been done in *Drosophila* by clone generation or RNAi experiments. However, generating a LOF situation requires some time to take effect in the cell; and secondary effects from the RNAi might hinder the protein's LOF phenotype. Various methods have been developed to try to overcome these problems, based on protein-targeted degradation or sequestering. One of these methods, named Anchor-Away (AA), has been established in yeast to generate LOF phenotypes by sequestering the target protein to another region in the cell. The main advantage of this method is that all the components are present in the cell, and it can be induced by the addition of rapamycin, being one of the fastest methods to deplete proteins in the cell. The AA method requires three components: a protein anchor, a target protein and rapamycin. The first one has to be an ubiquitous and largely present protein, either a membrane protein or a ribosomal unit, which is tagged with the human 12 kDa FK506 binding protein (FKBP12). The target protein is tagged with the 11 KDa FKBP12-rapamycin binding domain of the human TOR protein. If rapamycin is present, it drives the formation of a ternary complex that keeps the anchor and the target tightly bound. In our group, we are adapting the protocol to be used in *Drosophila melanogaster*, both *in vivo* and *in vitro*. We have successfully integrated all the components of the system in the fly, and we have checked that the anchor and the tagged protein are properly expressed and conserve their functions. For a pilot experiment, we have chosen Brinker, as it is a well studied protein and its LOF phenotypes are well described.

909B

Chemical control of gene expression in *Drosophila melanogaster*. Sachin Sethi, Jing W. Wang. Neurobiology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Spatiotemporal control of gene expression is an important approach to study biological mechanisms. In *Drosophila*, several techniques have been developed to temporally control gene expression using temperature (TARGET) or small molecules (GAL4-Geneswitch). While some of these techniques are incompatible with existing stocks of GAL4 lines, others suffer from the detrimental effects of high temperature on physiology and behavior. Here, we describe a post-translational method to control gene expression which can bypass the aforementioned problems. We have adopted a chemical method to regulate protein stability by fusing *Escherichia coli* dihydrofolate reductase (ecDHFR)-derived destabilizing domains (DD) to our protein of interest (Iwamoto et al, 2010). In the absence of trimethoprim (TMP), a stabilizing agent, this protein is targeted for degradation by the proteasome. To characterize the stabilization dynamics of this system *in vivo*, we fused GFP to DD and expressed it in the antennal lobe using the GAL4/UAS binary system. Flies fed with 1mM TMP showed intense GFP accumulation, more than 30 times greater than flies fed with DMSO, within 24 hours, in different olfactory neurons, ranging from peripheral sensory neurons to central mushroom body cells. Additionally, we could control the extent of this accumulation in a dose-dependent manner by varying the concentration of TMP fed to the fly. We demonstrate that this induction is reversible, as greater than 80% of the accumulated GFP could be degraded within 24 hours of removing flies from TMP. We did not observe any negative effect on the life span of flies fed on 1mM TMP when compared to those fed on DMSO. To our knowledge, this is the first chemical system in *Drosophila melanogaster* to confer post-translational control on gene expression. In summary, we can reliably control *in vivo* protein levels with negligible background, large dynamic range and rapid accumulation, all by feeding a small molecule to flies. .

910C

To Apply the CRISPR/Cas9 System in Drosophila. Jin Sun[1], Xingjie Ren[1], Zhihao Yang[1], Jiang Xu[1,2], Lu-Ping Liu[1,2], Jian-Quan Ni[1]. 1) School of Medicine, Tsinghua University, Beijing, China; 2) Tsinghua Fly Center, Tsinghua University, Beijing, China.

We have previously reported an effective and inexpensive CRISPR/Cas9 based method for genome DNA editing in *Drosophila melanogaster*. In this system, plasmids encoding sgRNAs are injected into embryos from germline specific Cas9 transgenic flies. However, single-guide RNA (sgRNA) parameters affecting the specificity and efficiency of the system are still not clear. Here, we found that off-target effects did not occur in regions of genomic DNA with three or more nucleotide mismatches to sgRNAs. In addition, we observed that the $Cas9^{D10A}$ nickase approach almost avoided off-target effects. Importantly, we documented a strong positive correlation between mutagenesis efficiency and sgRNA GC content of the six protospacer-adjacent motif-proximal nucleotides (PAMPNs). Further more, by injecting well-designed sgRNA plasmids at the optimal concentration we determined, we could efficiently generate mutations in four genes in one step. Our work demonstrates a comprehensive optimization of sgRNA and promises to vastly simplify CRISPR/Cas 9 experiments in Drosophila.

911A

Application of CRISPR/Cas9 system and efficient mutant screening in *Drosophila* species J.-S. Chen, **C.-T. Ting**. Dept Life Sci, National Taiwan Univ, Taipei, Taiwan, ROC.

Recent studies show that CRISPR/Cas9 system can be applied to generate deletion mutants in many organisms. However, all the reported applications in *Drosophila* are solely for the model species,*Drosophila melanogaster*. Here, we applied the CRISPR/Cas9 system one step further to *D. simulans*, *D. yakuba,* and *D. virilis*. For non-model species, lacking of useful genetics tools is the major difficulty to screen mutants. To circumvent the problem, we designed the double guide construct of a pair of gRNAs, with 500bp-1kb apart. A simple PCR based screen can be applied to injected funders given the high efficiency of CRISPR/Cas9 System. Using *muniature* as a test case, we systematically co-injected Cas9 and double guide gRNA vectors and detect the possible mosaic funders by PCR before screen the mutants through lengthy crosses. Our study demonstrates that CRISPR/Cas9 system can be a gene-engineering tool for non-*melanogaster* species.

912B

Fly Facility: A resource to facilitate modern molecular genetics in *Drosophila*. Deepti Trivedi. Fly Facility, Centre for Cellular and Molecular Platforms, Bangalore, India.

Drosophila has been a highly attractive eukaryotic model organism used to understand the functions of gene. Its adaptability from earlier approaches of gene modification techniques like mutations, insertions and deletions to recent genome engineering technologies used to precisely target a gene has made the fly a sought after model organism in the modern biology era. Over past 4 years fly facility at C-CAMP has provided services, including a reliable in-house fly stock maintenance service and an improved microinjection facility to generate transgene insertions, to several institutions worldwide. Our facility has embarked on CRISPR based gene editing that would enable precise genome editing and hence give a handle on the precise functions of genomic domains**.** We are also interested in providing genetic screening services and conducting courses within the facility.

913C

CoinFLP: A system for efficient mosaic screening and for visualizing clonal boundaries in *Drosophila*. Justin A. Bosch, Ngoc Han Tran, Iswar K. Hariharan. Molecular and Cell Biology, University of California - Berkeley, Berkeley, CA.

Screens in mosaic *Drosophila* tissues that use chemical mutagenesis have identified many regulators of growth and patterning. Many mutant phenotypes observed were contingent upon the presence of both wild-type and mutant cells in the same tissue. More recently, large collections of RNAi lines or cDNAs expressed under GAL4/*UAS* control have been used to alter gene expression uniformly in specific tissues. However, these newer approaches are not easily combined with the efficient generation of genetic mosaics. To address this we developed the CoinFLP system, a new genetic tool that uses a modified FLP/*FRT* recombination strategy and enables mosaic screens in the context of gene knockdown or overexpression. *CoinFLP-Gal4* generates mosaic tissues composed of clones where only a subset expresses Gal4. *CoinFLP-LexGAD/Gal4* generates tissues composed of clones that express either Gal4 or LexGAD thus allowing the study of interactions between different types of genetically manipulated cells. By combining *CoinFLP-LexGAD/Gal4* with the split-GFP system, GRASP, boundaries between genetically distinct cell populations can be visualized at high resolution. We demonstrate that these systems are easily adaptable to different tissues and cell types, and have validated this approach in the adult eye with an RNAi screen of genes that cannot be studied using the commonly used FLP/*FRT* stocks. Thus the CoinFLP system in *Drosophila* gives one greater control over gene expression in a mosaic tissue, visualization of cell contacts, and allows for more elaborate mosaic genetic screens.

914A

Resource Development for Investigation of Chromatin Modifying Proteins. Benjamin B Mills. Biology Dept., University of Alabama at Birmingham, Birmingham, AL.

Epigenetic modifications of DNA and histones are important for proper gene expression and regulation. Epigenetic writer proteins that accurately identify and modify target chromatin regions generate epigenetic marks. If these proteins do not function properly, errant gene expression occurs, potentially leading genome instability. Knowledge of how epigenetic writers function and how they are directed to their genomic targets is essential for our understanding of gene regulation. Histone methyltransferases (HMTs) are a class of

epigenetic writers that play an integral part in a variety of cancers such as prostate carcinoma, mixed-lineage leukemia, and breast cancer. Experiments performed in human immortal cell lines overexpressing certain HMTs such as G9a and SETD1B have shown that inhibiting these HMTs can lead to decreased cell proliferation. Thus, HMTs and likely other chromosomal proteins, have potential as therapeutic targets. However, our understanding of the molecular mechanisms that govern the actions and targeting of chromosomal proteins such as the HMTs is limited. Monoclonal antibodies are a valuable resource for the study of physical interactions between proteins and their localization. Therefore in collaboration with the *Epitope* Recognition and Immunoreagent Core Facility (*ERIC*), we are producing antibodies for the *Drosophila melanogaster* homologs of the human H3K9 HMTs as well as chromosomal proteins of the HP1 protein family. Short, recombinant proteins for *Drosophila* HMTs G9a, SU(VAR)3-9, and EGG have been expressed and purified. To date, three ELISA-positive hybridoma lines for G9a and nine for HP1B have been isolated. These antibodies are being screened for immunogenicity and specificity by Western blot and fluorescent *in situ* hybridization. Once fully characterized, these lines will provide a reliable, low cost source of antibodies for the study of H3K9 HMTs in *Drosophila*. The antibodies will facilitate the identification of protein interactors with HMTs as well as studies aimed at understanding their targeting to specific genomic regions. Resources made will aid future studies that have the potential to greatly improve our understanding of chromatin modifications.

915B

QuantiFly 4D: Learning to Count in 4 Dimensions. Richard M. Parton[1], Dominic Waithe[2], Lu Yang[1], Ilan Davis[1]. 1) Biochemistry Dept., University of Oxford, Oxford, United Kingdom; 2) Wolfson Imaging Centre, University of Oxford, Oxford, United Kingdom.

Brain development is an extremely complex 4D event that involves a vast number of cells, a large number of different cell types and takes place over a relatively long time course. The advancement in understanding brain development is hindered by difficulties in tracking events live and the lack of easily available, effective software for analyzing a tissue with such complexity. Over the last two decades,*Drosophila melanogaster* had proven to be an excellent model for understanding neurogenesis and stem cell proliferation and differentiation in mammals. However, for *Drosophila* and *vertebrae* alike, almost all experiments rely on antibody staining in fixed material for identifying different cell types and "snap shots" of the developing brain to understand cell kinetics. These approaches are both inefficient and inaccurate, they limit our ability to identify new mutations by means of high throughput screening projects and the accurate analysis of previously identified mutants. In the current study, we attempt to provide solutions to the problems outlined above by delivering a novel long term imaging method for imaging intact developing *Drosophila* brains in combination with a novel machine learning based algorithm called *Dynamic Brain*. *Dynamic Brain* software aggregates multi-channel 3D information into a simple centre-of-mass representation for each cell and so makes large scale cell counting for a dynamic developing brain simple and efficient. To achieve this, *Dynamic Brain* applies computer vision feature detection along with an ensemble of extremely random decision trees to characterize and organize the microscopy data. Through using the system it is possible to recognize, segment and count different cell-types based on their morphology and fluorescence distribution across multiple channels, something that has previously unachievable with existing thresholding techniques. *Dynamic Brain* provides experimental possibilities that were previously not achievable with currently existing methods for studying brain development and thus will accelerate the understanding of neural stem cell biology. .

916C

Creation of a *Drosophila* ORFeome library. Johannes Bischof[2], **Emma Sheils**[1], Mikael Björklund[1], Konrad Basler[2]. 1) University of Dundee, Dundee, United Kingdom; 2) Institute of Molecular Life Sciences, University of Zurich, Zürich, Switzerland.

Overexpression screens are regularly used to explore gene functions but until recently this strategy lacked a comprehensive and systematic collection with efficient methods for generation of such strains in Drosophila. In previous publication we have efficiently generated large numbers of transgenic Drosophila strains, and a collection of UAS-ORF fly lines that were created with the site-specific ΦC31 integrase method. Following the publication of our pilot study we have been working to extend the scope of our collection with the -addition of around three thousand Drosophila open-reading frames.

917A

Towards an automated experimental platform for *Drosophila melanogaster*. Matt Zucker[1], **Dave Zucker**[2]. 1) Swarthmore College, Swarthmore, PA; 2) FlySorter LLC, Cambridge, MA.

Combining robotics, automation, and machine learning can open up new possibilities for scientists working with fruit flies. The development of a fully automated experimental platform for *Drosophila* will enable high-throughput experiments and screens, and relieve researchers from many of the tedious and error-prone fly handling tasks. We demonstrate a robotic system aimed at solving key problems along the way to that goal, namely: imaging, classification and manipulation. Our primary contribution is a novel, two-stage method using computer vision and machine learning to analyze anesthetized flies in arbitrary orientations. First, each pixel in a reduced-resolution fly image is assigned a body part label (head, wing, body, leg, or background) using a two-layer neural network, with fewer than 8% of pixels mis-identified. This is more than sufficient to partition the original image into continuous regions by body part. The labeled fly image enables vision-guided manipulation of the fly, locating features with sub-millimeter accuracy. This labeled image can also subsequently be used to re-orient the original high-resolution image, allowing automatic extraction of a region of interest (ROI), targeting anatomy such as eyes, wings, and bristles. These extracted ROI images may be used as input to further classification steps; we demonstrate using them to sort flies by sex. Other novel aspects of our system include a simple yet functional vacuum-based manipulator and a low-cost robotic platform enabled by both rapid prototyping technologies and the open source 3D printing community. The device is capable of acquiring both coarse and high-resolution images of flies, manipulating them, as well as sorting them into vials by detected class. Finally, we conclude with preliminary results which address improving the speed, accuracy, and

generality of the proposed system, in order to advance the goal of producing a fully automated *Drosophila* lab.

918B

An LD-based approach for increasing coverage in pooled sequence experiments without additional cost. Hussein Al-Asadi, Matthew Stephens. University of Chicago, Chicago, IL.

Pooled sequencing is an increasingly popular technique for assessing genome-wide population allele frequencies. Fly stocks are sometimes documented in reference panels (such as the DGRP) which allows fly researchers to improve power in their study by using additional information from the reference panel. In human imputation studies, the panel is used to obtain correlation information between SNPs, and intuitively, can also be used to share information between reads to improve the precision of allele frequency estimates in pooled sequencing experiments. In this study, we demonstrate use of an LD-based model which incorporates correlation information from a panel to improve the precision of allele frequency estimates. The input to our method is the number of observed reads (per SNP) and the output is simply the *effective* number of reads (per SNP), leaving the data format unchanged. Thus, our approach allows researchers to increase their coverage without additional cost, and continue to use the same methods to analyze their data. We test our method on pooled DGRP flies using the DGRP as the reference panel. .

919C

A deep learning based universal representation for *Drosophila* Embryos Image Annotation. Qian Sun[1], Wenlu Zhang[2], Rongjian Li[2], Sudhir Kumar[3,4], Jieping Ye[1], Shuiwang Ji[2]. 1) School of Computing, Informatics, and Decision Systems, Arizona State University, Tempe, AZ.Biodesign Institute, Arizona State University, Tempe, AZ; 2) Department of Computer Science, Old Dominion, Norfolk, VA; 3) Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA; 4) Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia.

Automated and efficient software for analyzing images capturing spatial patterns of *Drosophila* gene expression is a prerequisite for generation of biological insights into gene functions, interactions and networks. A central theme in Drosophila embryo image annotation is to develop appropriate image representations for the specific task at hand. Texture features based on wavelets were particularly effective for determining the developmental stages from in situ hybridization (ISH) images. Such image representation is however not suitable for controlled vocabulary (CV) term annotation because each CV term is often associated with only a part of an image. Existing methods used handcrafted local features combined with high-level image representations to encode images for CV term annotation. We present problem-independent feature extraction methods that can be applied to a variety of different tasks. Our approach is based on the deep convolutional neural networks (CNNs) that can act on image pixels directly. To make the extracted features generic, the models were trained using a natural image set with millions of labeled examples. These models were then transferred to the ISH image domain and used directly as feature extractors to compute image representations for different tasks. We expect our features to be applicable to a variety of different tasks and evaluated them on two example applications. Experimental results showed that our deep learning based universal representations significantly outperformed problem-specific schemes in CV term annotation and achieved competitive performance on developmental stage prediction. Our work demonstrated that feature extractors trained on natural images can be used as generic feature extractors for different biological image informatics tasks.

920A

FlyBase: A new and improved website coming to a browser near you. Josh Goodman, Victor Strelets, Jim Thurmond, Gary Grumbling, Thomas Kaufman. FlyBase, Department of Biology, Indiana University, Bloomington, IN, USA.

We report on our new and improved web site that we will release in 2015. This upgrade will bring a number of changes both to the website itself and to the software powering it behind the scenes. For the overall website, we have updated the look and feel to improve general usability as well as how it performs on tablets at your bench or on your 27" display at your desk. The data reports for all our data types (genes, alleles, insertions, etc.) have general usability improvements and new features such as a sidebar navigation tool to help you locate your data of interest more quickly. FlyBase search results (HitLists) have been reworked to add smoother sorting, a cleaner look with more information, and filter capabilities, so you can quickly drill down to results that matter most to you. Moving HitLists between FlyBase tools or to your device is now easier with improved import/export and additional download options.
The FlyBase BLAST service has also been updated with the latest version of NCBI BLAST, an improved interface, and improved BLAST reports. For developers, we will be rolling out a new suite of web services that will let you query and pull FlyBase data in real time.
While there will be many changes, we are striving to keep things as familiar as possible to limit the time you need to invest in learning the new site. Your comments and suggestions regarding our website are always very appreciated.

921B

DRSC Informatics Tools for Functional Genomics Studies. C. Hu, I. Flockhart, C. Roesel, A. Vinayagam, B. Yilmazel, A. Comjean, L. Perkins, N. Perrimon, S. Mohr. Genetics, Harvard Medical School, Boston, MA.

Drosophila RNAi Screening Center, Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
A set of online informatics tools has been developed at *Drosophila* RNAi Screening Center (DRSC) to help scientists identify genes, select RNAi reagents, analyze high-throughput datasets and validate results. Here, we present recent updates to existing tools and new tools. DIOPT (flyrnai.org/diopt) was developed for query of predicted orthologs among 8 common model systems by integrating 10 ortholog prediction approaches. DIOPT-DIST (flyrnai.org/diopt-dist) facilitates human disease-relevant studies in model organisms based on disease gene annotation from OMIM and GWAS. UP-TORR (flyrnai.org/up-torr) was implemented for users to compare and

choose appropriate RNAi reagents from public resources. Our CRISPR sgRNA tools include a resource of pre-computed sgRNA designs viewed in a genome browse context, as well as annotation of potential off-target locations and predicted efficiency. FlyPrimerBank (flyrnai/flyprimerbank) is a genome-wide qPCR primer resource for*Drosophila.* Online GESS (flyrnai.org/gess) was implemented to evaluate potential off-target transcripts of siRNA/shRNA screens by seed-region analysis for any given species. Analysis of high-throughput data increasingly relies on pathway and functional annotation. To supplement existing tools and analyze network dynamics, we developed COMPLEAT (flyrnai.org/compleat), which facilitates high-throughput data mining and visualization and SignedPPI (flyrnai.org/SignedPPI/), which integrates PPI networks with RNAi phenotypes to reveal activation or inhibition of interacting protein pairs. We will present example workflows and practical tips to help researchers make the most of our online resources, as well as solicit feedback on how to further improve these resources.

922C

Gene Groups in FlyBase. Steven Marygold, Helen Attrill, FlyBase consortium. FlyBase, Dept. of Genetics, University of Cambridge, Cambridge, United Kingdom.

Many publications describe sets of genes that share a common biology. Here, we describe how FlyBase is curating such 'gene groups' in Drosophila, and how we are making these data accessible through new 'Gene Group Reports' on the website.
We define a 'gene group' simply as "a set of genes whose products are acknowledged to share a biological and/or molecular function", which includes evolutionary-related gene families and components of protein complexes. For example: histones, actins, GPCRs, myosins, proteasome subunits, ribosomal proteins, Wnts, cadherins. The composition of each group, and its organization into any subgroups, is manually curated from recent research papers or review articles. Comments are attached to the group where the membership is unclear or disputed, and a definition of the group is written based on the source references. This method ensures that FlyBase gene groups are of high quality and reflect the current knowledge in the published literature. Finally, key Gene Ontology (GO) terms that describe the group are applied, and links to orthologous gene sets at other databases are made. All this information will be presented on new 'Gene Group Reports' on the FlyBase website, which will also include buttons to easily access associated data, such as gene expression, phenotypes, or protein interactions. Whilst compiling each gene group, we also review the nomenclature and GO annotation of individual genes, improving consistency and accuracy where necessary/feasible. Our approach is thereby an efficient way to improve the quality of data at the level of individual genes, whilst simultaneously building a resource for the presentation and analysis of gene groups in FlyBase.

923A

UAS-driven Cerulean and Venus hybrid reporters provide calibration standards for FRET analysis in *Drosophila* tissues. K. Mecklenburg[1], H. Xia[2], S. Freed[3], J. E. O'Tousa[3], D. F. Ready[4]. 1) Indiana Univ. South Bend, IN; 2) Weill Cornell Med. Coll., NY; 3) Univ. of Notre Dame, IN; 4) Purdue Univ., IN.

The development of genetically encoded *Drosophila* FRET biosensors provides a powerful approach for measuring physiological changes in living animals. A diverse range of sensors have been developed, including those for Ca^{2+}, PKA activity, and cAMP. While these studies provide information within their individual paradigms, the adoption of a community-wide set of FRET standards would enhance our ability to compare FRET data between studies. Monomolecular FRET standards with a Cerulean (C) donor and a Venus (V) acceptor separated by 5, 17, and 32 amino acids with defined high, medium and low FRET efficiencies have been developed for evaluating FRET imaging for cells in culture. For standardizing FRET measurements in *Drosophila*, we generated stocks that express C, V, C5V, C17V, and C32V under Gal4/UAS control. To ensure similar stoichiometric expression levels independent of position effects, the C, V, and C-V standards were integrated into the same genomic site using the ΦC31 site-specific recombination system. These transgenic stocks are homozygous viable, thus allowing a variety of fluorochrome combinations to be generated by genetic crosses. For example, C and V placed in trans generates an animal in which the chromophores are not tethered and provides a control for measuring background FRET. To demonstrate the broad tissue and cellular applicability, we expressed these standards using muscle, nervous system, and photoreceptor drivers. Robust expression was observed in all tissues. Standard widefield and confocal fluorescence microscopes were used to image FRET emission. FRET efficiency was determined using the freely available ImageJ plug-ins RiFRET and FRETTY. Our results indicate that FRET efficiencies are similar to those obtained in cell culture systems. Our work therefore establishes standards for calibrating FRET measurements, and facilitates the adoption of FRET analysis in*Drosophila*.

924B

Targeted insertions in *Drosophila* cell lines using φC31 integrase. Lucy Cherbas[1], Jennifer Hackney[1,2], Lei Gong[1,3], Claire Salzer[1], Eric Mauser[1], Dayu Zhang[1], Peter Cherbas[1]. 1) Dept Biol and Drosophila Genomics Resource Center, Indiana Univ, Bloomington, IN; 2) School of Math and Nat Sci, New College, Arizona State Univ, Phoenix, AR; 3) Hangzhou School of Agr and Food Sci, Zhejiang Agr. and Forestry Univ, Zhejiang Lin'an, Hangzhou, China.

φC31 integrase-mediated targeted insertion or substitution into engineered docking platforms is now widely used in *Drosophila*. We sought to transfer this technology to established *Drosophila* cell lines, taking advantage of the wealth of well-characterized, diverse cell lines from *Drosophila* now available. We constructed docking sites for targeted substitution (RMCE), with and without insulator elements flanking an attP-bounded substitution site marked by a GFP expression cassette. Using P element transformation, single copies of these docking sites were inserted into the cell lines Kc167 and Sg4. We also constructed attB vectors with which constructs can be targeted to the docking sites; these vectors include a methotrexate-resistance marker between a pair of attB sites, to enable selection for cells which have incorporated the attB-bounded fragment, and a HSV-TK expression cassette outside the attB-bounded fragment, for ganciclovir

counter-selection against cells in which the entire plasmid has been incorporated by illegitimate recombination. We will describe protocols for targeted insertion into the docking sites, and present some examples of targeted transformants produced in this way. A few lines carrying targeted inserts were characterized, and their properties compared with clonal lines generated by a more traditional approach to stable transformation. Our work complements parallel efforts by Simcox and her collaborators who have established *de novo* cell lines from genetically characterized flies carrying φC31 docking sites.

925C

A comprehensive and precise genome duplication kit in *Drosophila*. Ming Fa, Min Li, Rui Chen, Graeme Mardon. GenetiVision Corporation, Houston, TX.

A fly stock collection carrying molecularly-defined genomic duplications is a very useful tool for complementation testing and genetic fine mapping. To improve upon and add to existing collections, a new set of transgenic flies that carry >80 kb BAC genomic clones covering the majority of the autosomal portion of the genome is being generated. Specifically, a set of 1,200 overlapping BACs that tile the entirety of chromosomes 2 and 3 was selected and are being inserted using site-specific integration. Once completed, together with the existing genomic duplication collections for the X and 4thchromosomes, more than 99% of the entire *Drosophila* genome will be covered. Due to the precise nature of this new collection where the end points of each BAC and the genomic insertion site are known, additional applications are enabled, such as clonal analysis. Furthermore, this collection can serve as the starting point for other manipulations such as gene tagging, generating point mutations, and structure-function analyses. To allow easy access to this collection, a web-based searchable database will be set up (http://genetivision.com/duplicationkit). Transgenic stocks will be made available as they are generated and the entire collection will be completed by the fall of 2015.

926A

An *in vivo* reporter system to trace aged cells in *Drosophila*. Xingchen Liu[1], Lei Liu[1,2]. 1) State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Peking University, Beijing 100871, China; 2) Beijing Institute for Brain Disorder and Beijing Tiantan Hospital, Capital Medical University, Beijing 100069, China.

Aging is an inevitable developmental process in most organisms. While, understanding the mechanism of aging at individual cell level is a fundamental theme in biological research. However, method to identify and trace aged cells *in vivo* is inadequate. To probe this question, we established an *in vivo* reporter system based on the phenomenon that proteins of more than 60Kd can only permeate through the nuclear membrane in aged cells. Thus, we generated two transgenes in *Drosophila*. One transgene expressed the DNA binding domain of Gal4 with its N-terminus tagged with HA, and C-terminus tagged with a leucine zipper domain (HA-Gal4-DB-LZ). The other transgene expressed the transcriptional activation domain of Gal4 with its N-terminus tagged with a different leucine zipper domain (Gal4-AD-LZ, its molecular weight is greater than 80kD). Both of these two transgenes were flanked with a heat shock promoter (*hs*) at their 5' ends. Their homozygous line was crossed with a *UAS-GFP* line. Upon heat shock to co-express the transgenes, two split parts of Gal4 interact at their leucine zipper domain and become functional to drive GFP expression. In the larval flies, we observed that the HA-Gal4-DB-LZ localized in the cytosol, suggesting the protein tags added to the Gal4-DB disrupted its nuclear targeting. As a bulky protein, Gal4-AD-LZ should not be able to enter the nucleus. Therefore, we expected the GFP expression only in the mitotic cells (the nuclear membrane disassembles during cell division) and the aged cells. Indeed, we observed strong GFP expression in the most active tissues of cell division such as the gut, but no or weak GFP signal in the muscle, fat body and tracheal cells. These results suggest that our reporter system works as designed. Then, we examined the GFP expression under the treatment of paraquat, as it introduces oxidative stress and may promote aging. As expected, we observed enhanced GFP expression in the guts and other tissues. Currently, we are examining whether this reporter system can trace individual aged cell in tissues during development.

927B

BioTapestry: Modeling Gene Regulatory Networks for Development. Suzanne Paquette, Kalle Leinonen, **William Longabaugh**. Institute for Systems Biology, Seattle, WA.

BioTapestry is a well-established tool for building, visualizing, and sharing models of gene regulatory networks (GRNs), with particular emphasis on the GRNs underlying development. BioTapestry was first used to model the sea urchin endomesoderm GRN in 2003, and has since been used across a wide variety of different model systems. It uses a hierarchy of models to present multiple views of the network at different levels of spatial and temporal resolution, and uses a visual representation that is tailored to the presentation of developmental GRNs. The new Version 7, released in September 2014, uses recent web browser technologies such as HTML5 Canvas to display the GRN model directly inside the browser. This now allows interactive graphical GRN models to be shared over the web without requiring the end user to have Java installed. At the same time, this new software architecture continues to support the traditional BioTapestry Editor Java desktop application that is used to build GRN models. We are also now beginning a project to support the ever-increasing complexity of developmental GRN models by making BioTapestry's model hierarchy more flexible and more scalable.

928C

Manipulation of Gene Expression by Infrared Laser Heat Shock and Its Application to the Study of Tracheal Development in *Drosophila*. Guangxia Miao[1,2], Shigeo Hayashi[1,2]. 1) Laboratory for Morphogenetic Signaling, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan; 2) Department of Biology, Kobe University Graduate School of Science, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo, 657-8051, Japan.

Induction of gene expression in a specific cell and a defined time window is desirable to investigate gene function at the cellular level

during morphogenesis. To achieve this, we attempted to introduce the infrared laser–evoked gene operator system (IR-LEGO, Kamei et al., 2009) in the *Drosophila* embryo. In this technique, infrared laser light illumination induces genes to be expressed under the control of heat shock promoters at the single cell level. We applied IR-LEGO to a transgenic fly stock, *HS-eGFP*, in which the eGFP gene is placed under the control of heat shock protein 70 promoter, and showed that eGFP expression can be induced in single cells within 1-2 hours after IR illumination. Furthermore, induction of *HS-Branchless* transgene encoding the *Drosophila* Fibroblast Growth Factor (FGF) effectively altered the migration and branching patterns of the tracheal system. Our results indicated that IR-LEGO is a promising choice for the timely control of gene expression in a small group of cells in the*Drosophila* embryo. By using IR-LEGO, we further demonstrated that the tracheal terminal branching program is sensitive to localized expression of exogenous FGF.

929A

New stocks at the Bloomington Drosophila Stock Center. Annette L. Parks, Kevin R. Cook, Thom C. Kaufman, Kathy A. Matthews. Bloomington Drosophila Stock Center, Dept. of Biology, Indiana University, Bloomington, IN.

The Bloomington Stock Center added over 4000 stocks in 2014, bringing our total number of stocks to over 53,000. In 2014 we added >1500 TRiP RNAi stocks, >1000 Mi{MIC} stocks and we completed acquisition of the Janelia lexA collection. We also brought in a variety of exciting new stocks, including cell cycle markers, tension sensors, red-shifted channelrhodopsins, gustatory receptor GAL4s, GTPase biosensors, pre- and post-synaptic terminal markers and more. We will provide an overview of these and other new acquisitions and highlight some important sets of pre-existing useful stocks. We hope we will give you ideas for experiments or new ways to explore biological processes. We welcome suggestions and questions, so come by and see us and let us know how we are doing.

930B

Evidence-Based Inquiry Into the Remote: Using *Drosophila's* Phenoloxidase to Open the Door to Research-Based Experiences in a High School Classroom. Rebecca Steiger[1], Nicole Green[2], Erika Geisbrecht[2], Carolyn Ferguson[3]. 1) Junction City High School, Geary Co. USD 475, Junction City, KS; 2) Bioch. & Mol. Biophysics Dept., Kansas State University, Manhattan, KS; 3) Biology Dept., Kansas State University, Manhattan, KS.

The advent of common core standards is driving secondary education curricula to include application of high-order skills in evidential and research based experiences. To meet these standards, students must be asked to learn in an open-inquiry, experimental-design setting. Although many classrooms rely on virtual experiences to allow students to gain exposure to current research, it should be argued that adoption of actual research experiences will yield the desired higher-order skills necessary to prepare students to be 21st century learners. In an effort to meet such needs, the NSF GK-12 funded program, EIDRoP (Evidence-based inquiry into the distant, remote, or past), pairs Kansas State University graduate students with a partner high school teacher to form teaching modules incorporating facets of student research to reinforce concepts and encourage student interest in STEM fields. The use of model organisms in the classroom setting provides a platform for designing open-inquiry research experiences. *Drosophila melanogaster* is used in this teaching module to examine protein folding and function. Using the well-characterized enzyme, Phenoloxidase (PO), students were introduced to the remote concept of enzyme functionality using assays to 'see' the effects of mutations and environmental variables on enzymatic activity. This module was an introductory lesson for students to engage in experimental design and understand protein macromolecules using standard research methods to assess melanization. Both qualitative phenotypic assays and a quantitative spectrophotometer experiment were performed by students in a standard high school classroom lab. Post-experimental analysis of data focused on troubleshooting and evaluating data against known literature and the development of technical writing through journal-style reporting. This module is part of a year-long collaboration introducing biochemistry and related fields using relevant research topics in *D. melanogaster*.

931C

The Council on Undergraduate Research- a resource for future faculty to integrate research and educational activities. Joyce Fernandes. Biology Dept, Miami Univ, Oxford, OH.

The mission of the Council on Undergraduate Research (CUR) is to support and promote high-quality undergraduate student-faculty collaborative research and scholarship. CUR has adopted five strategic pillars to aid the organization in planning and envisioning the future of undergraduate research. These pillars focus on areas that Councilors have deemed most important to the mission of the organization. They include: (1) Integrating and Building Undergraduate Research into Curriculum and Coursework. (2) Assessment of the Impact of Undergraduate Research (3) Diversity and Inclusion in Undergraduate Research. (4) Innovation and Collaboration in Undergraduate Research (5) Internationalization and Undergraduate Research. CUR's leadership works with agencies and foundations to enhance research opportunities for faculty and students. Other activities include support of faculty development through Institutes and Workshops (Institutionalizing Undergraduate Research, Initiating and Sustaining Undergraduate Research Programs, Proposal Writing, and Beginning a Research Program); Publishing books and articles related to creating, managing, and evaluating undergraduate research programs; Assisting administrators and faculty members in improving and assessing the research environment at their institutions; Providing information on the importance of undergraduate research to state legislatures, private foundations, government agencies, and the U.S. Congress. [The presenter is a Councilor in the Division of Biology, co-chair of the Organization's Advocacy Committee, and a Drosophila Biologist].

932A

An Undergraduate Course Combining Authentic Research and Scientific Writing. Sarah E. Hynek, Tomer Avidor-Reiss. Biological Sciences, University of Toledo, Toledo, OH.

Encouraging undergraduate students to pursue a career in science has posed a significant challenge for a number of years. Overcoming this requires that students will be confident in their capacity to be scientists. There are two components to creating confident young scientists: giving students a broad theoretical background via classes, and providing activities that apply and give real life meaning to this theoretical knowledge. However, student opportunities to perform research in a lab and taking part in the scientific endeavor are limited due to space in university research groups. This leaves many students completing their undergraduate degree without meaningful research experience. To overcome this, we have developed a course that is an intermediate between a standard "cookbook" style teaching lab, a writing intensive class, and research in a lab. This course combines scientific writing and "authentic research." Students who take the class learn how to write grants, advanced genetics, and perform authentic research in a *Drosophila melanogaster* teaching lab. Students take the knowledge that they gained in other lecture courses and apply it in a unique and hands on way. This course has been performed as a pilot in the summer of 2013 and the fall of 2013, and then as an official course in fall 2014. At present, 19 students have taken or are taking the course, 8 are still undergraduates, 6 have since joined a research lab as an undergraduate, and 3 others are pursuing higher education in biomedical fields. Based on the end of the semester evaluation of the pilots, all students indicated the course is satisfactory or more than satisfactory. About 30% thought it was better than most courses, and about 30% thought it was the best course in the university. This suggests the course, although challenging, provides a needed avenue to authentic research.

933B

An Inquiry Based Genetics Laboratory Utilizing Randomly Mating Populations of *Drosophila melanogaster*. M. Kimble, A. Schirmer, T. Campbell. Dept of Biology, Northeastern Illinois University, Chicago, IL.

Inquiry based learning, the integration of biology and math, and learning science by doing science are widely accepted as important components of a quality science education. However, designing and implementing these types of learning experiences can be challenging, especially when the exercises will be done in multiple laboratory sections taught by different instructors and where funds to support the labs and access to the teaching labs are limited. We have designed a lab activity where students analyze the phenotypic trends in randomly mating *D. melanogaster* populations. In brief, each student establishes a population of *D. melanogaster* composed of wildtype plus six mutant strains. The population is maintained through 3-4 generations. Phenotype data is collected at each generation and the population put through a bottleneck. After the final generation (F_3 or F_4) the data for all local (individual) populations is collected and entered into a single table showing the phenotypes and numbers of each present in each population and summed to form the global population. For the analysis, students are required to discuss changes that occurred in their local population over the course of the 3-4 generations, calculate allele frequencies for the six genes (starting mutant strains) in their local and global populations, and compare their local population to the other local populations. This exercise meets best practices in being an inquiry based, open-ended exercise with no specific expected results. The exercise integrates statistical analyses (allele frequencies, chi-square, and correlation analyses), and provides the students with a deeper understanding of the precepts of population genetics, genetic drift, and the Hardy-Weinberg Equilibrium. Preliminary data indicates a gain in student understanding of population dynamics and the Hardy-Weinberg Theorem. When combined with a formal write up, it also serves to strengthen student writing skills. In addition to meeting the above learning goals, the exercise is relatively inexpensive to conduct and does not require either students or the instructor to commit time outside of the scheduled class time to fly handling or data collection.

934C

The Genomics Education Partnership: Assessing and improving a research-based genomics project for undergraduates. D.W. Paetkau[1], J. Braverman[2], M. Burg[3], J. DiAngelo[4], C. Jones[5], K. Jones[6], L. Kadlec[7], N. Kokan[8], M. Manier[9], A. Nagengast[10], J. Sanford[11], K. Saville[12], C. Small[13], R. Spokony[14], J. Stamm[15], C. Ting[16], M. Wawersik[17], L. Zhou[18], W. Leung[19], C.D. Shaffer[19], S.C.R. Elgin[19]. 1) Saint Mary's Col (IN); 2) St. Joseph U; 3) Grand Valley St U; 4) Hofstra U; 5) Moravian Col; 6) Huntingdon Col; 7) Wilkes U; 8) Cardinal Stritch U; 9) George Washington U; 10) Widener U; 11) Ohio Northern U; 12) Albion Col; 13) Medgar Evers Col; 14) Baruch Col; 15) U Evansville; 16) National Taiwan U; 17) Col of William and Mary; 18) U Pittsburgh; 19) Washington U at St. Louis.

The Genomics Education Partnership (GEP) began in 2006 with 16 members interested in providing genomics research experiences to their upper division students. Since that time, the GEP has grown to a consortium of over 100 colleges and universities providing classroom undergraduate research experiences in bioinformatics for students at all levels. Attitude and knowledge assessments show that GEP students exhibit learning gains irrespective of the implementation strategy (e.g., short lab modules, entire courses, computer science/biology hybrid courses, or independent research projects). Both learning and attitudinal gains are strongly correlated with the amount of instructional time; students report gains commensurate to summer research experiences when substantial time is spent on the project (~36+ hours). Faculty assessment shows that barriers to the implementation of this research-based curriculum (e.g., campus acceptance of genomics in the curriculum, availability of IT/computer services, faculty expertise) can be alleviated through a central core facility that provides curriculum materials, wet lab and computational resources, collaborative pedagogy development, and a supportive community. GEP students are currently involved in the manual sequence improvement and gene annotations for the Muller F and D elements of eight Drosophila species. GEP faculty are also developing new curriculum materials to make the GEP research project more easily accessible to beginning students. The consortium is actively recruiting faculty interested in developing classroom research

experiences for first and second year students. Supported by HHMI grant #52007051, NSF grant #1431407 and Washington University at St. Louis.

LATE ABSTRACTS

935A

Fluctuation analysis of centrosomes reveals a suppressive role of Kinesin-1. Maheshwar Gummalla[1], Franziska Winkler[1], Lutz Kunneke[2], Zhiyi Lv[1], Annette Zippelius[2], Timo Aspelmeier[3,4], Jeorg Grosshans[1]. 1) Institute for Developmental Biochemistry, Medical School, Georg-August-University,Justus-von-Liebig Weg 11, 37077 Goetingen, Germany; 2) Institute for Theoretical Physics, Georg-August-University, Friedrich-Hund-Platz 1, 37077 Goettingen, Germany; 3) Institute for Mathematical Stochastics, Georg-August-University, Goldschmidtstr.7, 37077 Goettingen, Germany; 4) Felix Bernstein Institute for Statistics in the Biosciences, Georg-August-University, Goldschmidtstr.7, 37077 Goettingen, Germany.

The dynamics of the cytoskeleton is determined by filament turnover, filament associated proteins and molecular motors. On a time scale of minutes, the cytoskeleton and organelles are stable or undergo stereotypic movements, such as centrosome separation prior to mitosis. Little is known about the behaviour of the network on short time scales of seconds or subseconds. At these time scales, movement is driven by passive thermal fluctuations or by active processes arising from non-equilibrium dynamics of the cytoskeleton. These two types of motion can be assessed by a detailed analysis of the cytoskeletal movements and fluctuations. We recorded the movement of centrosomes, which are at the nodes of the microtubule network in syncytial Drosophila embryos at 1 Hz. First, by fluctuation analysis, we find that F-actin is required for directional movement during initial centrosome pair separation, whereas in latrunculin injected embryos the separation proceeds in a diffusive manner. Second, we focused on the trajectories of individ- ual centrosomes, calculating a fluctuation parameter as the deviation from temporally and spatially slowly varying drift movements. The fluctuations depended on the cytoskeleton, as they were suppressed both by cortical F- actin and microtubules. Surprisingly, the microtubule motor Kinesin-1 also suppressed fluctuations. Kinesin-1 may link astral microtubules to cortical actin and in this way stabilize the microtubule network. Consistent with this notion, we found that both Kinesin-1 and Dynein localized at the cortex and that cortical Dynein localization depended on Kinesin-1.

936B

Coordination of myosin pulses by transcription factor gradients. Natalie C. Heer, Adam C. Martin. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Morphogenesis is the process of reshaping tissues through the coordinated actions of individual cells. How cell behaviors are coordinated across a tissue and whether precise coordination is required for the formation of the final structure is poorly understood. During the early embryogenesis of the Drosophila embryo, the process of ventral furrow formation moves mesoderm precursor cells to the interior of the embryo. Ventral furrow formation provides an excellent system for studying the folding of epithelial sheets in the context of a living organism. Specifically, constriction of the apical domain changes cell shape from columnar to wedge-shaped, bending the epithelial sheet. This process is thought to be coordinated both by the induction of specific transcription factors in the ventral domain of the embryo as well as feedback between neighboring cells due to mechanical coupling of the tissue. The ventral domain of the embryo is specified by two transcription factors, Twist and Snail, which are required for apical constriction. Though Twist and Snail are expressed throughout the ventral domain, it has been shown that cells immediately along the ventral midline apically constrict earlier than more lateral cells. We hypothesize that different cell behaviors are due to different levels of Twist activity within the ventral domain. To this end we have quantified the expression of Twist and Twist target genes as a function of dorsal-ventral position in the ventral furrow. We have found that ventral cells express higher Twist levels than more lateral cells before the onset of gastrulation. However, Twist expression differences disappear over time, such that by the onset of ventral furrow formation, expression is uniform across the ventral domain. We have also quantified differences in myosin activity as a function of dorsal-ventral position to correlate differences in dynamic myosin activity with different histories of transcription factor expression. These results will clarify the mechanisms that coordinate contractile force generation during epithelial folding and invagination.

937C

Paternal Poc1 is Essential for the Function of Zygote Centrosome. Atul Khire, Sarah Hynek, Enrique Rodriguez, Tomer Avidor-Reiss. Biological Sciences, University of Toledo, Toledo, OH.

Centrioles are conserved microtubule-based organelles essential for cell division. In animals, during oogenesis, centrioles degenerate, and therefore, oocytes lack centrioles, and do not contribute any centrioles to the zygote Instead, it has been reported that in many animals, centrioles are inherited by the zygote from the sperm. However, in many other animals, a single functional centriole is observed. In sea urchins, frogs, and *C. elegans,* the sperm provides two centrioles. Recently, we showed that Drosophila zygote consists of two centrioles namely, GC and the PCL. Thus, the sperm provides both GC and PCL during fertilization. In addition to this, we also know that, centrosome is also essential for fertilization to occur. During fertilization, immediately after the sperm fuses with the oocyte, the it provides centrioles that recruit maternally contributed pericentriolar (PCM) proteins to form the zygote's centrosome This centrosome, that is found near the male pronucleus forms astral microtubules reaches out to the female pronucleus. Then, the female pronucleus migrates along the astral microtubules until it fuses with male pronucleus. Here, for the first time, we report that, centriolar protein Poc1 plays a role in fertilization of Drosophila. A point mutation in the Poc1 protein leads to reduce fertility in male flies. Further, we provide evidence that this reduced male fertility has two pronged effects on zygote development: 1) The male pronucleus fails to

recruit astral microtubules and thereby there is a failure of female pronucleus migration.2) We also see defect at the mitosis stage of embryo development, with majority of embryos having nonpolar spindle that has a GC and a 2nd centriole. We conclusively prove that mitotic defect in the embryos is due to failure of the PCL in contributing to aster formation, suggesting that paternal Poc1 is essential for PCL function in the zygote and thereby playing a major role in modulating the zygote function.

938A

The role of Psidin in phagosome maturation. Catherine Brennan, Kristofer Serrano, Mobina Roshandell. Department of Biological Science, Cal State University Fullerton, Fullerton, CA.

Phagocytosis is an ancient and evolutionarily conserved immune defense mechanism in which microbes are engulfed into membrane-bound compartments called phagosomes. The maturation of the phagosome into an acidic, hydrolase-rich organelle that destroys its contents depends on a partially understood sequence of interactions of the phagosome with endosomes and lysosomes, as well as the recruitment of many proteins to the phagosome. We reported *psidin* mutants as one of the first phagosome maturation mutants described in any organism: in *Drosophila* blood cells lacking *psidin* function, engulfed bacteria are not destroyed, but instead proliferate. Psidin has subsequently been found to have roles both in actin cytoskeleton stability and in N-terminal acetylation. Our goal is to characterize the role Psidin plays in promoting phagosome maturation, and in particular, to determine which of these two molecular roles is important to phagosome function. We report here our analyses both of the phagosome maturation defect in *psidin* mutant blood cells, using fluorescent reporters of phagosomal pH and Rab recruitment, as well as of the dependence of Psidin phagosomal roles on its actin-modulating vs. N-terminal acetylation activities.

939B

Subcellular localization of the Golgi kinase Four-jointed. Hodaka Shiraishi, Hirofumi Nonoyama, Yoko Keira, **Hiroyuki O. Ishikawa**. Graduate School of Science, Chiba University, Chiba-shi, Chiba, Japan.

Four-jointed (Fj) is a Golgi kinase that phosphorylates extracellular cadherin domains of the atypical cadheins of Fat and Dachsous. Fj localizes in the Golgi, and modulates Fat-Dachsous binding by Fj-mediated phosphorylation on their cadherin domains. The Golgi complex is a stack of cis-, medial-, and trans-cisternae, and the cisternae are present as dispersed stacks in *Drosophila* cells. To investigate the subcellular localization of Fj, we expressed Fj tagged with the V5 epitope (Fj:V5). Fj:V5 was localized to the medial-Golgi cisternae in cultured *Drosophila* S2 cells and cells of the imaginal discs of*Drosophila*. To identify domains required for the subcellular localization of Fj, we performed domain analysis of Fj. Fj consists of four domains including putative signal peptide, transmembrane domain, hinge region, and kinase domain. A secreted form of Fj (sFj:V5), which carries N-terminal BiP signal peptides for secretion and lacks its transmembrane domain, localized to the medial-Golgi. In addition, A kinase-dead mutant of Fj (Fj^{GGG}:V5) also localized to the medial-Golgi. These results suggest that the extracelular domain of Fj is required for its proper subcellular localization, and the subcellular localization is independent of its kinase activity.

940C

Genetic analysis of invasive pathways engaged by the EcR-coactivator protein Taiman. Phil K. Byun, Kenneth H. Moberg. Emory University, Atlanta, GA.

The transformation of non-motile epithelial cells to a migratory state plays a significant role in normal development and diseases such as cancer. During *Drosophila* oogenesis, a specialized group of cells termed border cells (BCs) acquire the ability to detach from their host epithelium and migrate through surrounding cells to the posterior end of the oocyte. The steroid receptor transcriptional co-activator*taiman* (*tai*) plays an important role in promoting this motility process, but its downstream transcriptional targets remain poorly defined. Here we introduce a novel, pathogenic model of Tai-driven tissue invasion that allows for rapid genetic screening for elements of the Tai-induced transcriptional program. Overexpression of *tai* in non-motile pupal wing cells causes these cells to invade through adjacent thoracic cuticle and into internal tissues, leading to a high-penetrance adult phenotype of wing tips embedded into the thorax. Using this phenotype as the basis for a dominant-modifier screen identified approximately 20 enhancers and suppressors. One of the strongest suppressors was found to correspond to alleles the *PRL-1* gene, which encodes a protein tyrosine phosphatase. Work by others has shown that *PRL-1* is transcriptionally induced by ecdysone in cultured S2 cells, and that a vertebrate homolog is overexpressed in metastatic colon cancers and supports metastasis in mouse models. Preliminary genetic and molecular analyses of *PRL-1* in our Tai-driven wing invasion model suggests that transcriptional induction of *PRL-1* by Tai is a key step in the acquisition of invasive ability among normally non-motile wing epithelial cells. Additional data on *PRL-1*, other modifiers identified in the genetic screen, and a recently completed RNA-sequencing analysis of Tai-induced mRNAs in wing cells, will be presented.

941A

The Role of *bric-à-brac 2*, a Transcription Factor, in Swarm Cell Migration in the *Drosophila melanogaster* Ovary. Aisha M. Dorta, Cassandra G. Extavour. Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

The intercalation and migration of cells are critical processes in the morphogenesis of many organs. For example ovarian development in *Drosophila* involves morphogenetic movements during the third larval instar that ensure that different populations of somatic cells occupy appropriate places within the ovary, and differentiate into the various component cell types of the functional adult ovary. One such movement serves to ensure the separation of anterior somatic cells, which become terminal filament cells, from posterior somatic cells, which give rise to the basal stalks. This separation begins with the posterior migration of differentiated somatic cells called swarm cells. Concurrently, terminal filament cells intercalate in the anterior region to form stack-like structures called terminal filaments (TFs).

We previously showed that swarm cell migration can be disrupted by knocking down *bric-à-brac2* (*bab2*) in the somatic cells of the larval ovary. This also led to an increase in the proportion of posterior somatic cells relative to anterior cells, resulting in fewer anterior cells and fewer TFs.
bab2 is a transcription factor which plays a role in ovarian morphogenesis, as does its paralog *bab1*. *bab1* is explicitly expressed in TFCs while *bab2* is expressed widely in somatic cells including swarm cells. Null *bab2* mutants are semi-sterile, while flies carrying null alleles of both *bab1* and *bab2* are completely sterile. Here we test the hypothesis that *bab2* plays a role in swarm cell migration, and perform experiments to distinguishing this putative role from the known role of *bab2* in TF morphogenesis.

942B
Role of Cdc42 in collective cell migration. Nathalie Colombié, Anne Combedazou, Valérie Cadamuro, Xiaobo Wang, **Damien Ramel**. LBCMCP UMR 5088 CNRS-University Toulouse III, Toulouse CEDEX 9, France.
Collective cell migration is critically important in physiological processes not only in physiology such as embryo development and homeostasis, but also in pathologies such as cancer. During Drosophila oogenesis, the so-called border cells perform a single stereotypic migration between germ cells toward the oocyte to form a future sperm-entry point. As a simple and genetically tractable in vivo model, border cell migration provides a good platform for our understanding of collective cell movement. Directional information of border cell movement is provided by ligands of receptor tyrosine kinases (RTKs). Activation of these receptors leads to the activation of the small GTPase Rac1, which is required to direct border cell migration. The role and the regulation of Rac start to be well documented in the context of collective cell migration. However, little is known about the implication of the other members of the Rho GTPases family. In particular, function of Cdc42 has not been extensively addressed despite its role in polarity and in cell migration in a wide range of system. Here, we demonstrate that Cdc42 is required for collective cell migration by participating in the regulation of protrusions restriction to the leading edge. Moreover, FRET measurement indicates that Cdc42 activity is uniformly distributed into the cluster. Finally, we demonstrate that Cdc42 act upstream of JNK signaling to control collective cell movements.

943C
A first phase of follicle elongation is driven by a double gradient of JAK/Stat activity. Herve Alegot, Cornelia Fritsch, Benoit Aigouy, Pierre Pouchin, Olivier Bardot, Vincent Mirouse. GReD Laboratory, Clermont-Fd (France), France.
The *Drosophila* ovarian follicle is used to study tissue elongation, one of the basic mechanisms of tissue morphogenesis. Current model proposes that extracellular matrix fibres, deposited during follicle rotations around their antero-posterior (AP) axis, act as a molecular corset constraining the mediolateral growth, thus promoting AP elongation. Preventing rotation or collagen deposition leads to round eggs. However we found out that in *Fat2* mutant follicles, where no rotation occurs, elongation takes place normally up to stage 7 meaning that rotation is dispensable for early elongation. We also observed that polar cells, found at the AP extremities of each follicle determine the elongation axis. Polar cells secrete the JAK/Stat pathway ligand Unpaired, establishing a double gradient as the follicle is growing. Both loss and gain of function experiments indicate that the spatial control of JAK-STAT activity controls early elongation. Live imaging of follicular cells reveals a pulsatory activity of their apical domain. This peculiar behaviour is correlated with the pattern of Stat activity. Thus we hypothesize that Stat activation triggers pulsations that are primarily seen all over the round follicles and these pulses then become restricted to the poles, acting as pulling forces inducing follicle elongation. We also observed oriented cell rearrangements correlated with follicle elongation, these rearrangements being likely induced by the pulling forces. This work is the first one that reveals how follicles elongate during the first stages. It also provides a mechanism explaining how a morphogen gradient can induce tissue elongation.

944A
Asl Localization During *Drosophila melanogaster* Spermiogenesis. Atul Khire, Alberto Vizuet Torre, Enrique Davila, Tomer Avidor-Reiss. Biological Sciences, University of Toledo, Toledo, OH.
Centrioles are conserved microtubule-based organelles that, together with pericentriolar material (PCM), form the centrosome. At the end of spermiogenesis, mammalian centrosomes undergo a process by which they lose their PCM and centriole structure is degraded, resulting in sperm with modified centrioles. Similarly, during *Drosophila* spermiogenesis, centriolar and PCM proteins are eliminated from the giant centriole (GC) and the proximal centriole-like (PCL), indicating that centrosome reduction also happens in *Drosophila*. The giant centriole marker PACT-GFP, which can be intensely observed in the spermatid giant centriole, is hardly observed in the spermatozoa giant centriole. To test if the PCL also undergoes centrosome reduction, and whether during centrosome reduction centriolar proteins are also eliminated from the giant centriole, we studied the localization of GFP-tagged centriolar proteins during spermiogenesis and in spermatozoa. We observed Ana1-GFP, BLD10-GFP, Ana2-GFP, Sas-6-GFP, Sas-4-GFP, and Asl-GFP in intermediate or round spermatids, in the giant centriole, and in the PCL. We found that all of these centriolar proteins are missing from mature spermatozoa, indicating centrosome reduction takes place in both the giant centriole and the PCL. This indicates that the PCL, which was formed during early spermiogenesis and lacks microtubules, is losing many of the proteins that formed it during centrosome reduction. To study Asl reduction, we analyzed Asl localization during spermatogenesis. Before meiosis, in spermatocytes, Asl labels the two connected GCs, labeling all along them. After meiosis and centriole separation, in round spermatids, Asl-GFP maintains the same labeling. As the spermatid differentiates, Asl localization around the GC shrinks gradually to a collar-like structure. Then, Asl is observed only in the PCL. Finally, Asl-GFP is eliminated altogether and cannot be observed in the spermatozoa. This localization is regulated differently by various Asl domains, suggesting that multiple mechanisms control Asl localization.

945B

Homologous recombination of *Drosophila* mitochondrial genomes. Hansong Ma, Patrick O'Farrell. Department of Biochemistry & Biophysics, UCSF, San Francisco, CA.

Despite the evolutionary role of homologous recombination in protecting genome integrity, it has not been experimentally demonstrated in the mitochondrial DNA of animals, leaving uncertain its contribution to mtDNA evolution, behavior of mitochondrial disease mutations, and somatic accumulation of age associated mtDNA damage. Here, by generating heteroplasmic *Drosophila* lines that allow selection for recombination between co-resident genomes, we recovered progeny rescued by recombinant genomes. In all four experimental settings, recombinant genomes took over. Introduction of double strand breaks markedly enhanced a low level of natural recombination in the germ line and appeared to promote somatic recombination. For a number of recombinants, we accurately mapped the exchange points by taking advantage of the high SNP density between pairs of diverged parental genomes, such as *D. melanogaster* and *D. yakuba* genomes. These findings experimentally document homologous recombination and its significance in metazoan mtDNA. In addition, detailed mapping provides initial characterization of the process. .

946C

Notch directly regulates genes involved in the control of glycolysis and the tricarboxylic acid cycle. Raquel Perez Gomez[1], Vera Slaninova[1,2], Michaela Krafcikova[3], Lukas Trantirek[3], Sarah Bray[4], Alena Krejci[1,2]. 1) University of South Bohemia, Ceske Budejovice, Czech Republic; 2) Biology Centre, Institute of Entomology, Ceske Budejovice, Czech Republic; 3) CEITEC, Masaryk University, Brno, Czech Republic; 4) University of Cambridge, Physiology, Development and Neuroscience, UK.

Rapidly dividing cells, such as tissues during development or cancer cells, initiate metabolic reprogramming towards increased glycolysis uncoupled from mitochondrial respiration, known as the Warburg effect. Notch signalling is known to stimulate cell growth in various contexts through the regulation of growth promoting transcription factors, diffusible signals and cell cycle regulators. Here we show that several genes involved in uptake of glucose, glycolysis, conversion of lactate to pyruvate and repression of TCA cycle are direct targets of Notch signalling that mediate cellular metabolic changes leading to Warburg effect. This model may provide a mechanism for Notch mediated cell growth during development as well as in Notch dependent cancers.

947A

Identification of Tribbles interacting genes using genetic and molecular approaches. L. Dobens, R. Das, A. Shipman. Sch Biological Sci, Univ Missouri, Kansas City, Kansas City, MO.

In humans and mice, mutations in Trb family members (Trb1, Trb2 and Trb3) are associated with both insulin resistance and cancer. In flies, Tribbles binds to and blocks the activity of key regulatory genes during diverse developmental processes, including (1) String/Twine/cdc25 phosphatase to restrict cell division in the embryo, (2) Akt1 to block cell growth in the larva and (3) the C/EBP homolog Slbo to modulate cell migration in the ovary. In mammals, Trb family members associate with an extensive list of proteins, including transcription factors, MAPKkinases and components of the spindle apparatus, but the importance of these associations is not entirely clear. To search for conserved Tribbles targets, we have used semi-automated software to analyze a high-throughput wing misexpression screen for genes that modify Tribbles tissue size and wing cell size phenotypes. Complementarily, we have developed Trbl antisera useful for immunoprecipitation approaches and have confirmed Trbl-Akt interactions in fat body extracts by Co-IP. We will present results from this ongoing work.

948B

Effects of *Wolbachia* on *Drosophila melanogaster* nutrition and microbiome. John Chaston[1], Adam Dobson[2], Angela Douglas[2]. 1) Brigham Young University, Provo, UT; 2) Cornell University, Ithaca, NY.

Many *Drosophila* are associated with the intracellular α-proteobacterium *Wolbachia*. Although best-known as reproductive parasite, *Wolbachia* has also been implicated in the regulation of iron homeostasis and host protection against many infections. The effects of *Wolbachia* on the gut microbiota and the wider nutritional biology of *Drosophila* and other insects have not been investigated. In this study, we investigated the relationship between *Wolbachia* presence and each of gut microbiota composition and nutritional indices (protein, triglyceride, glycogen and glucose contents) in *Drosophila melanogaster*. Using a subset of DGRP and other fly lines we demonstrate that both gut microbiota composition and host nutritional phenotypes vary depending on *Wolbachia* status of the host. These effects were host genotype-dependent (i.e. of varying magnitude and directionality), but we have not yet distinguished effects of genetic variation in the host or *Wolbachia*. Thus, the basis of these host genotype-specific effects of *Wolbachia* are likely complex, potentially including the multi-way interactions between the abundance and tissue tropism of the *Wolbachia*, *Wolbachia* genotype, host genotype and composition of the gut microbiota.

949C

The role of Drosophila adipocyte secretions in female fertility. S. Mosure[1], J. Sun[1,2]. 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Women with abnormal body fat content often experience irregular or absent menstrual cycles. This correlation suggests that fat tissue influences reproductive success, but the molecular mechanism that relates adiposity and infertility remains unclear. Data from our lab and others suggests that many aspects of reproduction are conserved between Drosophila and mammals, so we used Drosophila as a model to study the regulation of reproduction. Here, we investigated the role of Drosophila fat body secretions in regulating female fertility. Using two different fat body Gal4 drivers and tubGal80ts, we knocked down coat proteins involved in anterograde (Sec23 and

Sar1) and retrograde (bCOP) ER-Golgi vesicle transport in the adult fat body. We found that while all three coat proteins are required in the fat body for normal egg-laying, they are not required for sperm storage in the spermathecae or seminal receptacle. Sar1-RNAi causes widespread nurse cell apoptosis in vitellogenic egg chambers, suggesting that Sar1 is necessary for the ecdysone checkpoint at stage 8 of oogenesis. bCOP of the COP I protein complex is not required for oogenesis, but it is necessary for oviposition, the release of a mature egg from the uterus onto the egg-laying surface. Sec23 does not appear to be involved in either oogenesis or oviposition. Our findings suggest that each component of the secretory pathway may have a different role in regulating fertility. Further study will identify the processes associated with the observed phenotypes.

950A

Rescuing the individualization defect of *mulet* using the UAS/GAL4 system: A work in progress. James Fabrizio, Kavita Bharrat, Rachel Daniel, Iryna Koziy, Vincent Lombardo, Dwaine Pryce. Natural Sciences, College of Mt St Vincent, Bronx, NY.

Spermatogenesis in *Drosophila melanogaster* takes place in a common cytoplasm, and spermatozoa are ultimately resolved from this syncytium post-meiotically during spermatid individualization. An F-actin based organelle known as the Individualization Complex (IC) mediates the maturation of sperm from the syncytium, and these ICs are severely disrupted in *mulet* mutant testes indicating that *mulet* is required for individualization. *mulet* encodes the Tubulin-binding cofactor E (TBCE)-like protein, suggesting a role for microtubule dynamics in *Drosophila* spermatid individualization. Indeed, a population of ~100 inter-flagellar microtubules fails to disappear in *mulet* mutant testes just prior to individualization, suggesting that removal of these microtubules is necessary to maintain the structure of the IC(Fabrizio et al., 2012). Most recently, we have detected TBCE-like protein in elongated spermatid cysts, consistent with the results of Nuwal et al., 2012. Now, we are attempting to rescue the *mulet*mutant phenotype by overexpressing wild-type TBCE-like in the germline of mutant testes. To this end, we have obtained two GAL4 drivers (Hsp70-GAL4 and tubulin-GAL4) and confirmed that they drive expression in the germline, including late stage spermatogenic cysts. We have also successfully overexpressed SMN-tagged TBCE-like in the germline using these drivers. We are in the process of making the appropriate fly stocks so that UAS-SMN-TBCE-like can be overexpressed in the germline of hemizygous *mulet* mutant males. We expect to rescue the *mulet* mutant phenotype by overexpressing SMN-tagged TBCE-like using both GAL4 drivers. In the future, the same GAL4 drivers will be used to generate RNAi in the germline in order to phenocopy the *mulet* mutant and confirm the results of the rescue experiments.

951B

Genes expressed in early oogenesis are enriched in putative DSX targets and are important for ovary development. Hina Sultana, Haiwang Yang, Hangnoh Lee, Brian Oliver. NIDDK, NIH, Bethesda, MD.

Germline sex determination requires non-autonomous signals from the soma that are regulated by Doublesex (DSX), a conserved transcription factor that controls sex determination in many species. These signals may be produced in the somatic cells in the female germline niche. To characterize transcription in the niche, we performed 12 biological replicate experiments on the anterior and compared expression to whole ovary. We found 1262 genes with anterior ovariole biased expression and asked which were occupied by DSX in DamID-seq experiments on ovaries. Remarkably, 58% of genes with anterior ovariole biased expression are occupied by DSX (Fisher's Exact Test, $p < 2.2x10^{-16}$). We performed loss-of-function study using RNAi-mediated knockdown of 38 such genes in a subset of somatic niche cells with *traffic-jam* gal4 and found three genes that are specifically required for female but not male gonad development.

952C

Regulation of the germline stem cells maintenance by the chromatin remodelling factor ISWI. Maria Toto, D. Corona. Telethon Dulbecco c/o Univerisity of Palermo, Dpt STEBICEF, Edificio 16-Palermo Italy.

The germline stem cells (GSCs) of *Drosophila melanogaster* ovary provide an excellent model system to study the molecular mechanisms of stem cell self-renewal. The balance between stem cell self-renewal and differentiation is precisely controlled in order to ensure tissue homeostasis and to prevent tumorigenesis. The regulation of stem cells maintenance depends on changes in chromatin organization and tissue-specific transcriptional regulators. *In vivo* studies have shown that in Drosophila ovary the ATP-dependent chromatin-remodelling factor ISWI maintains germline stem cells in a single niche. However the exact role of chromatin remodelling in stem cell niches is poorly understood. To dissect the role of ISWI-mediated chromatin remodeler in controlling stem cell self-renewal, I developed a strategy to purify a large numbers of pure GSCs from the Drosophila ovary. Using this approach I generated a genome-wide transcriptome and chromatin-binding profile of ISWI on GSCs chromatin. To identify the potential regions of the genome that are bound by ISWI in GSCs, I conducted a ChIP-Seq analysis and I found nearly 7000 ISWI bound coding genes. Moreover, RNA-Seq experiments conducted in ISWI mutant GSCs revealed ISWI as major regulator of its target genes in GSCs. Furthermore, by motif discovery analysis I idenified, for the first time, a ISWI binding motif in GSCs termed GC-rich. ISWI binding element (GC-ISWI). I also found that GC-ISWI sequence had relatively affinity for Mad transcription factor. Furthermore, I characterized GSCs-specific ISWI target genes enriched in the GC-ISWI element genes by gene ontology analysis and I found specific gene networks under the control of ISWI to prevent premature stem cell differentiation. Our data suggest that the ATP-dependent chromatin remodeler ISWI works as a master regulator of a network of genes regualting GSCs self-renewal in the Drosophila ovary.

953A

Characterization of the hematopoietic response to tissue injury. Cory Evans, Ting Liu, Utpal Banerjee. Dept MCD Biol, Univ California, Los Angeles, Los Angeles, CA.

The *Drosophila* hematopoietic system has many important roles facilitating organismal development and mediating protective inflammatory responses, particularly the provision of cellular innate immunity upon infection by pathogenic organisms. Less is known about how *Drosophila* blood cells respond to inflammatory signals caused by tissue injury. It has been previously shown that tissue injury is sufficient to cause the differentiation of specialized blood cells known as lamellocytes, however the molecular mechanism underlying this process has not been described. Here we analyze the systemic blood response to distal epithelial injury. Consistent with previous findings, we find that injury-induced lamellocyte differentiation indeed occurs. Furthermore, we identify several signaling pathways activated in blood cells by the injury stimulus, and describe a mechanism causing the differentiation of lamellocytes.

954B

Amino acid-stimulated tolerance of *B. cepacia* infection of *Drosophila* is TORC-independent and facilitated by host microbiota. V. Allen[1], R. O'Connor[1], C. Zhou[1], V. Hill[1], E. Stone[1], R. Park[2], K. Murphy[3], J. Canman[4], W. Ja[3], M. Shirasu-Hiza[1]. 1) Department of Genetics and Development; Columbia University Medical Center; New York, NY, 10032; USA; 2) Department of Neuroscience; Dartmouth College; Hanover, NH, 03755; USA; 3) Department of Metabolism and Aging; The Scripps Research Institute; Jupiter, FL, 33458; USA; 4) Department of Pathology and Cell Biology; Columbia University Medical Center; New York, NY, 10032; USA.

There are two broad categories of defense against bacterial infection: resistance mechanisms to control microbial growth and tolerance mechanisms to withstand the pathogenic effects of infection. The metabolic state and nutrient intake of an organism, both of which are circadian-regulated, can affect both resistance and tolerance but dissecting their individual contributions during infection is complex and the underlying molecular mechanisms are not well understood. Our study of the *Drosophila Period* mutant led us to identify a novel link between increased feeding behavior and increased host tolerance against infection by *B. cepacia*, a bacterial pathogen of rising importance in hospital-acquired infection. We further found that infection tolerance in wild-type animals is stimulated by transient exposure to dietary glucose and amino acids. Glucose-stimulated tolerance is mediated by the insulin-like signaling pathway and can be induced by feeding or direct glucose injection. Using glucose injections, we identified a narrow window for induction of tolerance centered around the time of infection. In contrast to glucose, amino acids cannot be injected but must be ingested to stimulate host tolerance against infection. While the TOR signaling pathway is a canonical sensor of dietary amino acids, amino acid-stimulated tolerance unexpectedly appears to be TOR-independent. We further find that amino acid-stimulated tolerance is facilitated by the host gut microbiota. This work demonstrates that the nature, quantity, and timing of dietary intake on the day of infection by *B. cepacia* can make a significant difference in long-term survival and that host microbiota play an important role in facilitating amino acid-stimulated tolerance of infection in *Drosophila*.

955C

Development of a multicolor cell labeling system. Christopher K Roblodowski[1], Qi He[2]. 1) Biological sciences and Geology, Queensborough Community College, Bayside, NY; 2) Biology, Brooklyn College, Brooklyn, NY.

How neural circuits process information to control animal behavior is a fundamental issue in neuroscience. Neural circuitry formation during development consists of many steps including cell type specification, lineage differentiation, neural connectivity, synapse formation and production of different neurotransmitters A better understanding of neural circuitry is critical for analyzing the onset and progression of neural degenerative diseases such as Parkinson's disease. An obstacle in disease related research is a lack of more efficient cell marking methods to enable the tracing of cell lineage and neural cell-cell interaction. We have proposed a strategy for the development of a new multicolor cell labeling system (MULA) for detecting neural cell lineage and cell interaction. It utilizes the high efficient recombination system of PhiC31 and attB/attP coupled with fluorescent proteins of different colors. The availability of this method and the coupling of this method with other existing ones such as MARCM will offer a new investigative route for probing lineage and cell-cell interactions.

956A

The role of *drpr* in the clearance of degenerating dendrites. Maria L Sapar, Chun Han. Weill Institute for Cell and Molecular Biology, Dept of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Neurons exist in microenvironments where they rely on extrinsic and intrinsic factors to maintain homeostasis during development and after physical injury. The dendrites of sensory neurons degenerate as part of the remodeling program of neural circuits during an insect's metamorphic transition from larva to adult. Physical injury of dendrites also induces degeneration distal to the injury site. In both cases, neuronal debris must be recognized and cleared efficiently by phagocytes to prevent inflammation and maintain a healthy environment. In *Drosophila*, epidermal cells are the primary phagocytes responsible for clearing dendrite debris in the periphery nervous system after dendrite pruning or injury. Draper (Drpr) is a membrane receptor that plays a key role in engulfment and clearance of degenerating dendrites. Studies done with a $drpr^{\Delta 5}$ mutant that has parts of its 5'UTR and exon 1 deleted showed that loss of *drpr* function causes engulfment and clearance defects. However there is a difference in the extent of these defects between dendrite pruning and dendrite injury models. In the injury model, loss of *drpr* blocks engulfment and the dendrite debris retain the original dendrite pattern long after injury. Loss of *drpr* during dendrite pruning has a weaker phenotype where engulfment is only partially blocked and dendrite debris is eventually cleared. As $drpr^{\Delta 5}$ has the majority of the *drpr* locus intact, we set out to learn whether the weaker phenotype during dendrite pruning is caused by incomplete loss of *drpr* function or a redundant pathway for engulfment. We

used the CRISPER/Cas9 system to generate a*drpr* mutant with an indel mutation that resulted in a coding frame-shift across the whole locus, making it a true null mutant. To confirm the engulfment defects, we labeled dendrites with a membrane-associated pH sensor marker (MApHS), which changes color upon engulfment due to a pH drop in the phagosome. Our *drpr* null mutant has a similar phenotype as the $drpr^{\Delta 5}$ mutant. These results show that Drpr is not solely responsible for engulfment during dendrite pruning, which suggests that there is a redundant pathway mediating the engulfment and clearance of dendrite debris.

957B

Glycosphingolipid mannosyl glucosylceramide (MacCer) promotes neuromuscular junction growth in *Drosophila*. Yan Huang, Sheng Huang, Sin Man Lam, Zhihua Liu, Guanghou Shui, Yong Q. Zhang. Chinese Academy of Sciences, Beijing, China.

Lipids are major components of cellular membranes and play a fundamental role in a living organism. Mutations in lipid biosynthesis result in many diseases including intellectual disabilities (ID). For example, mutations in acyl-CoA synthetase long-chain family member 4 (ACSL4) are associated with non-syndromic ID. We previously reported that *dAcsl*, the *Drosophila* ortholog of mammalian *ACSL3*and *ACSL4*, inhibits neuromuscular junction (NMJ) growth probably by attenuating bone morphogenetic protein (BMP) signaling via endocytic recycling of BMP receptors. However, how lipids affect neural development and function remains poorly understood. From a genetic screen, we identified that mannosyl glucosylceramide (MacCer), a class of glycosphingolipids, is a positive regulator of larval NMJ growth and bouton formation. Biochemically, MacCer regulates association of Wingless (Wg), the ligand of Wnt signaling, with lipid rafts composed of sphingolipids and sterols, thereby affecting the activity of Wnt signaling and synaptic growth. Lipidomic analysis revealed an increased level of lipid raft-associated MacCer, ergosterol and phosphoethanolamine ceramide (PE-Cer, the *Drosophila*analogue of sphingomyelin) in *dAcsl* mutant brains. Importantly, reducing MacCer or sterol by genetic and pharmacological means rescued the NMJ overgrowth in *dAcsl* mutants, suggesting a critical role for lipid rafts in the pathogenesis of ACSL4-related ID. Together, our results reveal a crucial role for lipid rafts in synaptic development.

958C

Microtubule-severing protein Katanin regulates neuromuscular junction development and dendritic elaboration in Drosophila. Chuanxi Mao[1,2], Ying Xiong[2], Shan Jin[1], Qifu Wang[2], Yong Q. Zhang[2]. 1) Hubei University; 2) chinese academy of sciences, Beijing, China.

Microtubules (MTs) are crucial for diverse biological processes including cell division, cell growth and motility, intracellular transport and the maintenance of cell shape. MT abnormalities are associated with neurodevelopmental and neurodegenerative diseases such as hereditary spastic paraplegia. Among many MT regulators, katanin was the first identified MT-severing protein, but its neuronal functions have not yet been examined in a multicellular organism. Katanin consists of two subunits; the catalytic subunit katanin 60 contains an AAA (ATPases associated with a variety of cellular activities) domain and breaks MT fibers while hydrolyzing ATP, whereas katanin 80 is a targeting and regulatory subunit. To dissect the in vivo functions of Katanin, we generated mutations in Drosophila Katanin 60 and manipulated its expression in a tissue-specific manner. Null mutants of Katanin 60 are pupal lethal, demonstrating that it is essential for viability. Loss-of-function mutants of Katanin 60 showed excess satellite boutons, reduced neurotransmission efficacy, and more enlarged cisternae at neuromuscular junctions. In peripheral sensory neurons, loss of Katanin 60 led to increased elaboration of dendrites, whereas overexpression of Katanin 60 resulted in the opposite. Genetic interaction analyses indicated that increased levels of MT acetylation increase its susceptibility to Katanin-mediated severing in neuronal and non-neuronal systems. Taken together, our results demonstrate for the first time that Katanin 60 is required for the normal development of neuromuscular synapses and dendrites.

959A

The NAV2 homolog Sickie regulates F-actin-mediated axonal growth in *Drosophila* mushroom body neurons via the non-canonical Rac-Cofilin pathway. T. Abe, D. Yamazaki, S. Murakami, M. Hiroi, Y. Nitta, Y. Maeyama, T. Tabata. IMCB, the university of Tokyo, Tokyo, Japan.

During brain development, neurons undergo multiple morphological changes to form an elaborate neural network. Among various regulators of neuronal morphogenesis, Rac GTPase (Rac) and Cofilin [Tsr in *Drosophila*] play key roles in the axonal development. The Rac-Cofilin signaling pathway is essential for cytoskeletal remodeling to control the axonal growth of *Drosophila* mushroom body (MB) neuron. Rac canonically signals through the downstream Pak-kinase and LIM-Kinase to suppress the Cofilin-dependent axonal growth and also signals through a Pak-independent, non-canonical pathway to promote outgrowth. However, whether this non-canonical pathway converges to promote Cofilin-dependent Filamentous-actin (F-actin) reorganization in axonal growth remains elusive. We demonstrate that Sickie, a human microtubule-associated protein Neuron-navigator-2 (NAV2) homolog, cell-autonomously regulates the axonal growth of *Drosophila* MB neurons via the non-canonical pathway. Sickie was prominently expressed in the newborn, F-actin-rich axons of MB neurons. A *sickie* mutant exhibited MB axonal growth defects, and its phenotypes were rescued by exogenous expression of Sickie in young neurons. We observed phenotypic similarities and close genetic interactions among Sickie and Rac-Cofilin signaling components. Using the MARCM technique, distinct F-actin and P-Cofilin patterns were detected in developing axons mutant for *sickie* and Rac-Cofilin signaling regulators. We also found that the P-Cofilin levels were increased in *sickie* mutant brains, and upregulation of Cofilin function alleviated the axonal defect of the *sickie* mutant. Epistasis analyses revealed that Sickie suppresses the LIMK overexpression phenotype Slingshot (Ssh) phosphatase-dependently and is required for functions of the Pak-independent Rac1 and Ssh to counteract LIMK. Finally, we also found the genetic interactions among Sickie and microtubule components, EB1 and β-tubulin. We propose that Sickie controls axonal growth by regulating F-actin remodeling via the non-canonical Rac-Ssh-Cofilin pathway and microtubule dynamics.

960B

The effect of developmental exposure to nicotine on TH and dopamine expression in *Drosophila melanogaster* . Melanie C Morris, Norma A Velazquez-Ulloa. Biology, Lewis & Clark College, Portland, OR.

Although addiction to nicotine is fairly common, the biological mechanisms underlying this addiction are relatively unknown. In this study, we examined how developmental exposure to nicotine in*Drosophila melanogaster* affects the expression of the neurotransmitter dopamine in the adult fly brain. Chronic exposure to nicotine in other model organisms has previously been shown to increase the expression of tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, and we expected to see a similar effect in the fruit fly (Ferrari et al. 2011) . We used immunostaining techniques to mark TH in the dopaminergic neurons of fly brains and used staining brightness to approximate the relative amount of dopamine present. Imaged brains displayed previously identified dopaminergic neuronal clusters around the mushroom body known as PAM and PAL clusters (Mao and Davis, 2009). We compared the images and the brightness of the TH staining in fly brains that had developmental exposure to nicotine and those that had not to see if nicotine produced any observable changes in TH expression. These comparisons have provided a better understanding of how TH and relative dopamine expression is affected by developmental nicotine exposure.

961C

***Drosophila* models of Ret fusions in Papillary Thyroid Carcinoma. Sarah Levinson**, Ross Cagan. Dept. of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY.

Ret fusion proteins, including PTC1 (CCDC6-RET) and PTC3 (NcoA4-RET), drive a subset of Papillary Thyroid Carcinomas (PTC). There is currently no treatment for refractory, metastatic PTC, which results in 1500 deaths in the US each year. A challenge in identifying targeted therapies for Ret fusion driven cancers has been the lack of predictive models. We created transgenic, over-expression*Drosophila* models of PTC1 and PTC3, and found that they cause cancer phenotypes such as delamination, cell migration, and EMT. We conducted a genetic screen of the *Drosophila* kinome (252 alleles) and a drug screen of FDA approved cancer therapeutics (62 compounds) against flies expressing either PTC1 or PTC3. We found that PTC1 and PTC3, despite containing identical Ret kinase domains, have a different signaling output and are sensitive to different kinase inhibitors. Out of the 19 kinases that were identified as hits from the PTC3 kinome screen, only 5 overlap with PTC1 hits. PTC3 is most sensitive to Ponatinib, where as PTC1 is most sensitive to Cabozantonib. These findings suggest that patients may greatly benefit from targeted treatment directed against the specific Ret fusion that they harbor.

962A

Molecular and Genetics Screens Identify Genes Required for Developmental Ethanol Response in *Drosophila*. Chih Ching Wu, Janet Lafler, Payam Khodabakhshi, Rachael French. Biological Sciences, San Jose State University, San Jose, CA.

Fetal Alcohol Spectrum Disorder (FASD) describes a collection of physical and neurobehavioral disabilities brought on by maternal alcohol consumption during pregnancy. While it has been clear for over two decades that genetic factors are involved in the susceptibility to, and severity of, FASD, no genes mediating these effects in mammals have been identified. In addition, very little is known about the targets of ethanol during development, in flies or mammals. *Drosophila* larvae reared in ethanol mirror the detrimental effects of FASD; we are therefore using flies as a genetic model to identify developmental ethanol targets. In order to identify the genes involved in larval response to ethanol exposure in an unbiased fashion, we carried out two complementary screens. First, we generated approximately 1000 novel transposon-induced mutations and screened them for altered survival and development time when reared in ethanol. We have generated 30 new mutations, most of which are in genes previously unknown to be affected by (or to affect the results of) developmental alcohol exposure. Second, we carried out a microarray analysis to identify transcripts whose expression was altered in ethanol-reared 3rd instar larvae. Among the mutations we identified two alleles of *withered (whd*), the *Drosophila* homolog of carnitine palmitoyltransferase I (CPT1). These alleles are sensitive to the effects of ethanol, as are several previously existing alleles of *whd*. Additional mutations, including one in the *Drosophila* carnitine transferase (CT) gene, are also predicted to disrupt fatty acid metabolism, leading us to propose disrupted fatty acid metabolism as a potential mechanism for ethanol-induced developmental damage. This is an exciting finding, because several human developmental disorders, including Tay-Sachs disease and Niemann-Pick Disease, result from abnormal lipid metabolism. This presents the exciting possibility that some aspects of FASD may be treatable or preventable through simple dietary changes. We will present the findings of both screens and our plans for future characterization of the genes identified.

963B

Nucleolar stress due to loss of Nopp140. Allison James, Yubo Wang, Patrick DiMario. Biological Sciences, Louisiana State University, Baton Rouge, LA.

Metazoan development requires large numbers of functional ribosomes to support protein synthesis demands in rapidly dividing cells. Precursor ribosomal RNAs (pre-rRNAs) synthesized in the nucleolus undergo precise post-transcriptional cleavages and chemical modifications in forming mature 18S, 5.8S, and 28S rRNAs. These rRNAs assemble with ribosomal proteins to form small and large ribosomal subunits. “Nucleolar stress” results when ribosome biogenesis is halted altogether or when the assembled ribosomes are dysfunctional. Historically, *bobbed* mutations in *Drosophila* block ribosome biogenesis, while *Minute* mutations affect individual ribosomal proteins. Both lead to developmental abnormalities such as shortened bristles, malformed wings, legs, and cuticle, and slowed development; different tissues are particularly sensitive to the loss of functional ribosomes during their development. Our

current research focuses on Nopp140, which functions as a ribosome assembly factor, specifically a chaperone for snoRNPs that catalyze nucleotide-specific 2′-*O*-methylation and pseudouridylation of rRNA. We knocked down Nopp140 using gene knock-out and/or ectopic RNAi expression. Here we survey several nucleolar stress phenotypes caused by the loss of Nopp140 in different *Drosophila* tissues. Partial depletion of Nopp140 by RNAi expression in larval imaginal wing discs caused a vestigial wing phenotype in adults. Immunofluorescence microscopy with an antibody directed against cleaved caspase 3 showed apoptosis in the diploid larval wing disc cells. Interestingly, a *p53* gene deletion failed to rescue this phenotype, thus implicating a p53-independent apoptotic pathway. Other phenotypes associated with the loss of Nopp140 include the presence of electron-dense granules within the cytoplasm of stressed cells, autophagy in polyploid larval cells, and overexpression of Coilin. RT-PCR data indicate elevated *puckered* expression in *Nopp140-/-* larvae, strongly suggesting that JNK acts as a central stress effector during nucleolar stress. We conclude that loss of ribosome biogenesis in *Drosophila* induces p53-independent, but JNK-dependent nucleolar stress responses.

964C

Characterization of the motorneuron secretory route in a *Drosophila* model of hereditary spastic paraplegia. Cristian de Gregorio[3], Patricio Olguín[2], Andrés Couve[1,3], **Jimena Sierralta**[1,3]. 1) Program of Physiology and Biophysics, Faculty of Medicine, Universidad de Chile, Santiago, Metropolitan, Chile; 2) Program of Human Genetics, Faculty of Medicine, Universidad de Chile, Santiago, Chile; 3) Biomedical Neuroscience Institute, Faculty of Medicine, Universidad de Chile.

Hereditary spastic paraplegias (HSP) is a group of genetically diverse disorders characterized by spasticity and weakness of the lower limbs as a result from length-dependent axonopathy of the corticospinal tracts. Three genes encoding membrane-shaping proteins of the endoplasmic reticulum (ER) cause nearly 65% of HSP cases. Atlastin, the second most common HSP gene, is localized mainly to the tubular ER, where it mediates the homotypic fusion of ER membranes. Manipulation of Atlastin expression generates ER structural changes, but their consequences in the motorneurons function are not clear. Here we establish a *Drosophila* HSP model based on Atlastin downregulation, and characterize the organization of the secretory route components and synaptic proteins in motor neurons *in vivo* in these flies.

We generated flies with genetic downregulation of Atlastin in motor neurons and we assessed the locomotor behavior in larvae and adult flies. *HSP* larvae exhibited decreased crawling speed and contraction frequency, while adult flies showed reduced climbing ability. We evaluated the organization of the ER and Golgi apparatus (GA) in motor neuron somas and axons in flies that express RFP-KDEL or RFP-GalT. Both organelles showed altered morphologies in our HSP model. We are evaluating the axonal trafficking of membrane associated synaptic proteins such as Bruchpilot-GFP and synaptotagmin-GFP. Atlastin downregulation results in behavioral features of HSP, stressing the importance of this protein in motor neuron function. Moreover Atlastin regulates ER and GA morphology in somas and axons, validating our model as an attractive system to characterize HSP genes in locomotion function and protein trafficking. Conicyt USA20130020/ICMP09015F.

965A

Elucidating Mechanisms of Gene Expression Regulation by Survival Motor Neuron. T Yokokura[1], A. Fonseca[2], H. dos Santos[2], S. Yoshikawa[1], T. Chobanyan[1,3], H. Goto[1], M. Gama Carvalho[2], D. Van Vactor[1,3]. 1) OIST, Onna, Okinawa, Japan; 2) Bio-FIG, Faculty of Science, University of Lisbon, Lisbon, Portugal; 3) Dept. Cell Biology, Harvard Medical School, Boston, MA.

Spinal Muscular Atrophy (SMA) is a devastating inherited disorder characterized by progressive loss of motor activity due to death of motor neurons, failure of neuromuscular synapses and degeneration of muscles. Although SMA has been linked to mutations in *Survival Motor Neuron 1*(*SMN1*), molecular mechanisms underlying SMA are not yet well understood. Mutations in *Drosophila Smn*, sole fly ortholog of human *SMN1*, results in loss of peripheral axons, degeneration of muscles and defects in the formation and function of the neuromuscular junction (NMJ). In earlier genetic studies, lethality and NMJ phenotypes caused by *Smn* hypomorphic mutation can be modified by mutations in components of signaling pathways downstream of two trans-synaptic factors: Bone-morphogenetic protein (BMP) or basic-Fibroblast growth factor (FGF). In addition, *Smn* protein interactome analysis suggested that some of those canonical pathway components are conserved in the extended human SMN genetic network. Based on these findings, we asked whether reduction of *Smn* expression levels influences on expression of BMP- and FGF-signaling pathway components. We conducted qRT-PCR assays targeting common regions throughout isoforms of the genes in each pathway and found no significant changes in steady state mRNA levels induced by reduction of Smn levels. As Smn contributes to snRNP biogenesis, we then asked if reduction of Smn perturbs expression levels of any specific splice isoform of each gene. After testing 39 out of 49 isoforms for 17 genes in the two pathways using isoform-specific qPCR assay, we found that expression of two isoforms of *trio* (downstream of the BMP receptor Wishful thinking (Wit)) and one isoform of *stumps* (downstream of the FGF receptor Heartless) were altered by either neuron or muscle-specific knockdown of *Smn*. Furthermore, ongoing RNA-seq analysis of the Smn model appears to corroborate our qRT-PCR results. Thus, reduction of Smn is likely to perturb gene expression in a highly isoform-specific manner.

966B

Evolutionary dynamics of a developmentally constrained trait: Segment allometry across 12 *Drosophila* species. Gizem Kalay, Joel Atallah, Austin M. Tang, Mohan K. Murugesan, Amanda E. Crofton, **Susan E. Lott**. Evolution and Ecology Dept, University of California, Davis, Davis, CA.

The study of the evolution of development has often focused on fast evolving traits of relatively simple genetic basis. However, many of the developmental traits most critical to the fitness of the organism are complex, and under high levels of developmental constraint. Here, we present Drosophila larval segmentation as a model system to investigate how a complex and critical morphological trait

evolves. At the embryonic stage, segment allometry (the size of segments relative to body size) has been examined in a few species, and was found to be invariant within, but variable between species. In order to systematically and precisely quantify patterns of segment allometry, we developed a high throughput imaging and analysis pipeline. We have used this pipeline to extract position information from 1st instar larvae, across 12 species spanning the *Drosophila* genus. Using larval denticle belts as segment markers, we have found that there is considerable variation in segment allometry between the 12 *Drosophila*species. In general, we found that the anterior most abdominal segments are the most variable. For instance, the first abdominal segment is located more anteriorly in *D. melanogaster* and *D.virilis* as compared to the other species, including close relatives of *D. melanogaster*. The amount of variation in segment position decreases after the 3rd abdominal segment between all species, but *D. mojavensis*.*D. mojavensis* is less similar to all other species with a significant anterior shift in most of its segments. So far, our data suggests that this complex, developmentally constrained trait shows significant differences between closely related species. There is no strong phylogenetic signal in our data, suggesting that this trait may evolve through large phenotypic changes on some lineages, rather than through the continuous buildup of small phenotypic changes over evolutionary time. Further studies will pursue the genetic causes underlying differences in segment allometry between species, as well as the impact of the environment on this critical, highly constrained, developmental trait.

967C

The role of ecology and development in the evolution of female reproductive capacity in Hawaiian *Drosophila*. Didem Sarikaya, Cassandra Extavour. Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Animals evolving on volcanic archipelagos often undergo adaptive radiation, where traits are rapidly diversified in response to the new environments. Hawaiian *Drosophila* represent a group of close to 1000 species that radiated from a last common ancestor within the last 25 million years, and have adapted to specialize on decaying flowers, leaves, fungi, sap fluxes, and bark of native plants as egg laying substrates. Interestingly, Hawaiian Drosophila have the most extreme range of female reproductive capacity reported in the genus *Drosophila*. This diversity of reproductive capacity is the result of the fact that Hawaiian *Drosophila* have the widest range of the number of ovarioles. Ovarioles are the egg-producing strands that make up the ovaries, and ovariole number in this group ranges from two to 101 ovarioles per ovary depending on the species. Previously, it has been suggested that flies that laid eggs on more ephemeral food sources, such as flowers and leaves, have fewer ovarioles compared to species that laid eggs on less ephemeral food sources such as bark. To test this hypothesis using modern phylogenetic methods, we sampled over 100 wild-caught females representing species that occupy all known ecological niches. We previously identified two distinct developmental mechanisms involving one cell type, terminal filament cells (TFCs), that determined ovariole number during the larval stage in African *Drosophila* species. We obtained larval tissue from 21 species that represent flower, leaf, sap and bark breeders, and identified terminal filament cell number as the principle developmental mechanism that generated variation in ovariole number in Hawaiian *Drosophila*. We are developing these species as a novel model to investigate how female reproductive capacity evolves in response to ecological niche.

968A

Preliminary data on drosophilid – yeast interaction from a Neotropical forest fragment. M.R.D. Batista[1], R.D. Chaves[1], F.S. Uno[1], R. Tidon[2], C.A. Rosa[3], L.B. Klaczko[1]. 1) Depto. Genética, Evolução e Bioagentes, Inst. Biologia, Universidade Estadual de Campinas - UNICAMP, Cx. Postal 6109, Campinas, 13083-970 SP, Brazil; 2) Inst. Ciências Biológicas - GEM, Universidade de Brasília, CP 04457, Brasília, DF, Brazil; 3) ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

We carried out an experiment to characterize *Drosophila* species attraction to different yeasts at an Atlantic Rainforest fragment. After 48 hours growing in GYMP broth, we inoculated two different yeast species, *Hanseniaspora uvarum* and *Saccharomyces cerevisae* in autoclaved mashed banana and left to ferment for about 20 hours. Then, we exposed sets of three different baits, distant 5 meters from each other, in the field: non-inoculated autoclaved banana, autoclaved banana inoculated with *H. uvarum* and autoclaved banana inoculated with *S. cerevisae*. We collected drosophilids using entomological nets over fermented banana baits separatedely and brought them to the laboratory to be analysed. The flies were identified by their external morphology; genitalia of the wild males; and genitalia of the laboratory reared F1 males of collected females. Uninoculated banana baits (controls) attracted virtually no flies (11 versus 292 in the other ones; less than 5% of the total). Thus, the results from these flies were no longer analysed. Our results show that different drosophilids are preferencially attracted by the two yeast species (X^2 = 44.97; d.f. = 2; p < 0,001). Flies of the *tripunctata* group were mostly attracted to baits inoculated with *S. cerevisae* (92 in a total of 123 = 74.8%); while flies of subgenus *Sophophora* were mostly attracted to *H. uvarum* (66 in a total of 79 = 48.2%). Exotic species (*D. immigrans; D. simulans; D. suzukii; Z. indianus*) showed a clear preference for *H. uvarum* over *S. cerevisae* (only one male on this species baits in a total of 19) .Further investigations to characterize seasonal and intraspecific variation in food preferences are under way. Acknowledgement: Claudete Couto, Klélia Carvalho and Renato Cavasini for technical assistence. Funding Agencies: CAPES, CNPq, FAEPEX-UNICAMP, FAPESP, FAPEMIG.

969B

Transcriptional cofactor CDK8 modulates developmental transitions through the Ecdysone Receptor in *Drosophila*. Xiao-Jun Xie[1], Fu-Ning Hsu[1], Xinsheng Gao[1], Wu Xu[2], Yue Xing[1], Yani Zheng[1], Jian-Quan Ni[3], **Jun-Yuan Ji**[1]. 1) Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX; 2) Department of Chemistry, University of Louisiana at Lafayette, Lafayette, LA; 3) Gene Regulatory Laboratory, School of Medicine, Tsinghua University, Beijing 100084, China.

Ecdysone and Ecdyone Receptor (EcR) play critical roles in orchestrating the developmental transitions of arthropods. In *Drosophila*, EcR heterodimerizes with Ultraspiracle (USP) and directly controls the expression of many genes required for regulating developmental transitions. However, exactly how EcR-USP activates gene expression remains unknown. We have found that CDK8 and Cyclin C (CycC),

two subunits of the transcriptional cofactor Mediator complex, modulate developmental transitions by regulating EcR-activated gene expression in *Drosophila*. We observed that the homozygous*cdk8* and *cycC* mutant animals resemble the EcR mutants in both pupal morphology and the larval-pupal transition. Our genome-wide gene expression profiling analyses have revealed a systematic down-regulation of EcR-target genes in *cdk8* and *cycC* mutants. Although the levels of nuclear EcR and Ultraspiracal (USP) are elevated, EcR-USP binding to the polytene chromosome and the promoters of EcR target genes is diminished in *cdk8* or *cycC* mutants. CDK8-CycC interacts with EcR-USP *in vivo*, and CDK8 directly interacts with the AF1 domain of EcR. These results suggest that CDK8-CycC modulates the developmental transitions in *Drosophila* by regulating EcR-dependent transcription.

970C

Pan-leg developmental regulators control pro-thoracic leg specific Scr expression. Christopher McCallough, Ece Eski, Emily Wyskiel, Teresa Orenic. Dept Biological Sci, Univ Illinois at Chicago, Chicago, IL.

The Drosophila adult has one pair of legs on each of its three thoracic segments (T1-T3). Although these structures exhibit serial homology, the legs from different segments have distinct morphological features. One such feature is the patterning of the small mechanosensory bristles (mCs), which are components of the peripheral nervous system. In the T2 leg these mCs are organized into a series of longitudinal rows (L-rows) along the circumference of the tibia and tarsal segments. However, at specific positions along the circumference and proximal/distal (P/D) axis of the T1 leg, the L-rows are replaced by a group of mCs organized into transverse rows (T-rows) [1,2]. Studies have indicated that the position of T-row bristles on the tibia and basitarsus of T1 legs is established as a result of Hox gene alteration of the L-row patterning pathway [3,4]. In T1 prepupal legs, Sex combs reduced (Scr) is expressed at elevated levels within the T-row primordia. We have found that Scr modifies the mC pattern on T1 legs via repression of Delta, a key regulator of leg mC patterning [4]. Our model for T-row patterning suggests that a central step in this process is establishment of spatially defined Scr expression within defined domains of the leg primordia in response to the global regulators of leg development. The mechanisms that generate morphological diversity among the legs will therefore require an understanding of the regulation of Scr in the T-row primordia. Here we present our genetic studies on the regulation of Scr by genes known to pattern the leg along its circumference and P/D axis.

971A

Defining the functional domains of a LEM-domain protein Otefin. Wenfan Ke, Kaylee Lovander, Lacy Barton, Pamela Geyer. Biochemistry, The university of Iowa, Iowa city, IA.

The nuclear lamina is an extensive protein network that contributes to nuclear structure and function. One family of nuclear lamina proteins is the LAP2-Emerin-MAN1-domain (LEM-D) family. These proteins share a ~40 amino acid domain that binds the chromatin interacting protein, Barrier-to-Autointegration *F*actor (BAF). Drosophila has three nuclear lamina LEM-D proteins, named Otefin (Ote), Bocksbeutel (Bocks), and dMAN1. Both Ote and Bocks are homologs of human emerin, while dMAN1 is a homolog of human MAN1. Although these Drosophila LEM-D proteins are globally expressed, loss of either Ote or dMAN1 causes tissue-specific adult defects that differ from each other. Loss of any two of the three LEM-D proteins causes lethality. The reason for the shared and unique functions of LEM-D proteins is unknown. Here, we describe studies focused on Ote. Loss of Ote causes female sterility due a lack of survival of germline stem cells, while loss of Ote and either Bocks or dMAN1 causes lethality. We are defining critical functional domains of Ote. This protein carries an amino terminal LEM-D and a carboxyl terminal peripheral localization domain that separate a largely unstructured middle domain. To identify important functional regions with the unstructured domain, we aligned Ote from the twelve drosophilid species. These studies revealed nine conserved regions ranging in size from 6 to 26 amino acids with conservation extending between 56% and 87% similarity. To test the importance of these regions, we are generating transgenes that express mutant Ote proteins that carry deletions and/ or alanine substitutions of a single conserved region within the full length protein. We are testing the ability of each mutant protein to rescue $ote^{-/-}$ female sterility and $ote^{-/-}$, $bocks^{-/-}$ lethality. These studies will advance our understanding how LEM-D proteins contribute to nuclear lamina functions required for Drosophila development. .

972B

Genome Analysis of a Phylum: Progress on the I5K Pilot and towards a finished *D. pseudoobscura* at the Baylor College of Medicine Human Genome Sequencing Center. Stephen Richards[1], Shwetha Murali[1], Daniel Hughes[1], Shannon Dugan[1], Kim Worley[1], Stephen Schaeffer[2], Richard Gibbs[1]. 1) Human Genome Sequencing Center, Dept of Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Biology, The Pennsylvania State University, University Park, PA.

The i5K is an initiative to sequence the genomes of 5,000 arthropods of medical, agricultural and scientific importance. To identify challenges in species selection, identification and acquisition, DNA isolation, sequencing strategies and assembly, automated and manual annotation, analysis and publication, the BCM-HGSC is sequencing ~30 arthropods, selected by the i5K species selection committee. We present our progress, working with 30 groups of collaborators on the project, the lessons from our attempts to obtain DNA, sequence and assemble multiple genomes in a factory environment. We also present our automated annotation pipeline based on the Maker pipeline, but uses extensive metazoan protein alignment evidence, RNAseq evidence from the species being sequenced, as well as automatic training of the ab-initio Augustus and SNAP gene predictors. Approximately 30 evidence tracks are additionally generated for manual annotators using the web-Apollo tool, enabling manual annotators to see all input evidence into a gene model. To date 28 species have been put through the i5K pilot annotation pipeline, and are being hosted by the National Agricultural Library providing access to search, community manual annotation and browser functionality. We discuss the challenges of scientific analysis of this many genomes, the co-ordination of publications, and what questions can be answered looking at the genomics of a phylum.

FInally, we working to significantly upgrade the D.pseudoobscura genome with 70X PacBio de-novo assemblies, (725kb contig N50) new Illumina assemblies (2 Mb scaffold N50), HiC chromatin seq data, and BioNano genome mapping. Data is available pre-publication, and we are working to combine these datasets to get as close as possible to a finished genome reference.

973C

Automatic *Drosophila* egg chamber stage identification from DAPI image. Dongyu Jia[1], Qiuping Xu[2], Qian Xie[3], Washington Mio[2], Wu-Min Deng[1]. 1) Department of Biological Science, Florida State University, Tallahassee, FL; 2) Department of Mathematics, Florida State University, Tallahassee, FL; 3) Department of Statistics, Florida State University, Tallahassee, FL.

The *Drosophila* egg chamber is an excellent and well established model in developmental biology. Its development is divided into 14 stages. Biologists determine the specific stages of the egg chamber based on egg chamber morphology and some stage-specific markers in their routine work. However, visual stage determination is a very tedious, subjective and time-consuming task. This study presents an objective and reliable method for quantifying cell features and classifying cell stages based on DAPI image. The proposed approach is composed with two steps: a cell feature extraction step and a statistical modeling step. The cell feature extraction step measures cell size, oocyte size, distribution of follicle cell. A feasible method of determining the on-site of polytene stage and the centripetal migration is also discussed. The statistical modeling step uses linear and ordinal regression for exploring the stage-features relationship and classifying cell stages. Additionally, user intervention is also allowed when the automatically learned parameters can not help to provide a proper decision. Around 200 images were used for training and evaluating the method. Cell area is a stable feature for stage differentiation, while oocyte size becomes useful for late stage differentiation. This method combined with machine learning algorithm has great potential to discover hidden developmental mechanisms.

974A

Discovery-based science education: *Drosophila* functional genomics approaches in the undergraduate laboratory. Cory Evans, John Olson, Ira Clark, Utpal Banerjee, UCLA Undergraduate Research Consortium for Functional Genomics. Biomedical Research Minor, MCD Biol, Univ California, Los Angeles, Los Angeles, CA.

We have utilized large-scale functional genomics projects as a tool to introduce early undergraduates to the research laboratory experience. Here we describe two of these projects and how students contribute original research. We also describe assessment data summarizing the impact of this research experience.

975B

From mutant gene to behavior and back to gene: genetic screening for high-school students, non-biology majors, and biology majors. Norma Velazquez Ulloa[1], Jennifer Lakeman[1], Melanie Morris[1], Ariel Shaw[1,2]. 1) Biology, Lewis and Clark College, Portland, OR; 2) Cleveland High School, Portland, OR.

Very few opportunities exist for collaborative research between high school and undergraduate students. Biology faculty at Lewis and Clark College incorporate their scientific research into the classroom in introductory and advanced courses. Here we present an example of student research involving a high school summer research intern and her undergraduate mentor, non-majors in a lower-division biology course (BIO100: Perspectives in Biology), and students in an upper division biology course (BIO380: Behavioral Genetics). Students in the BIO100 course (Spring 2014) pioneered a genetic screen for nicotine and ethanol sensitivity. Students worked in groups, and were assigned a fly strain whose survival after nicotine exposure was known, or a mutant fly line with an unknown P-element insertion. Students exposed the flies to either nicotine or control food during development and assessed developmental delay, survival, and ethanol sedation. The data collected by these non-biology major students showed trends for either an effect of nicotine exposure or ethanol sedation. A high school summer intern carried out the next stage of the screen with supervision from an undergraduate mentor. The high school student re-analyzed the raw data from the BIO100 course, chose the most promising mutants to validate the initial trends, and helped optimize the experimental design. A new round of screening was completed by the BIO380 course (Fall 2014). These students completed the screen, from developmental exposure to behavior during the first half of the course, and carried out independent projects on the second half. As part of the screen students froze some of the leftover flies, so the summer students and the next BIO380 course can determine where the P-element insertions are and find the genes that influenced the phenotypes tested during the genetic screen. This work was supported by the M.J. Murdock Charitable Trust, the Miller Foundation, and the Community Engagement & Leadership in Science Miller program at Lewis and Clark College.