Full Abstracts

1 A novel Hdac1/Rpd3-poised circuit balances continual self-renewal and rapid restriction of developmental potential during asymmetric stem cell division D. Janssens[1,2], D. Hamm[5], L. Anhezini[1], Q. Xiao[1,3], K. Siller[6], S. Siegrist[6], M. Harrison[5], *C-Y. Lee*[1,2,3,4]. 1) Life Sciences Institute; 2) Cellular and Molecular Biology Graduate Program; 3) Department of Cell and Developmental Biology; 4) Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 5) Department of Biomolecular Chemistry, University of Wisconsin Madison, WI; 6) Department of Biology, University of Virginia, Charlottesville, VA.

Tissue-specific stem cells divide asymmetrically to self-renew while producing uncommitted intermediate progenitors that rapidly acquire restricted developmental potential. How the developmental competency of uncommitted intermediate progenitors becomes restricted in a timely manner remains completely unknown. We previously showed that *earmuff* (*erm*) uniquely functions to restrict the developmental potential of uncommitted intermediate progenitors (immature INPs) in the fly larval brain. Here, we elucidate a novel Hdac1/Rpd3-dependent mechanism that poises *erm* expression in neural stem cells (neuroblasts), and allows activation of *erm* expression in immature INPs within two-hours of their birth. Contrary to expectation, the histone deacetylase Rpd3, but not PRC2, prevents premature activation of the poised *erm* immature INP enhancer in self-renewing neurobalsts. The transcriptional repressors that promote self-renewal directly bind and function cooperatively through Rpd3 to maintain the *erm* immature INP enhancer in an inactive but poised state. In parallel, neuoblast-specific transcriptional activators bind and maintain the *erm* immature INP enhancer in an active state likely through multiple histone acetyl transferases. Down-regulation of transcriptional repressor activities in the immature INP provides the permissive cue to activate the poised *erm* enhancer, triggering Erm expression. Erm acts as a negative feedback mechanism, restricting the developmental potential of INPs by repressing genes encoding neuroblast transcriptional activators. This work provides a novel paradigm, in which removal of transcriptional repressors, rather than addition of a transcriptional activator, rapidly activates a poised regulator of the developmental potential in intermediate progenitors. We propose poising the expression of master regulators of differentiation through active histone deacetylation in stem cells maintains continual self-renewal while enabling rapid restriction of developmental potential following asymmetric division.

2 Hedeghog and Insulin Balance Proliferation and Autophagy to Determine Follicle Stem Cell Lifespan *Alana O'Reilly*[1], Tanu Singh[1,2]. 1) Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA; 2) Molecular and Cell Biology and Genetics Program, Drexel University College of Medicine, Philadelphia, PA.

Egg production declines with age in many organisms, a process that is linked with stem cell loss. Stem cells are maintained by locally produced signals and by systemic signals that mediate tissue responses to environmental cues. Together, these signals control stem cell self-renewal and generation of differentiated daughter cells for long-term tissue maintenance. Diet-dependent signaling has emerged as a critical mechanism for stem cell regulation, as caloric restriction or reduced insulin signaling can lengthen stem cell lifespan and delay aging. Epithelial Follicle Stem Cells (FSCs) in the fly ovary are exquisitely responsive to dietary changes, entering quiescence in the absence of food and initiating proliferation rapidly upon feeding. The proliferative response depends on two diet-dependent signals, cholesterol-mediated release of the growth factor Hedgehog (Hh) and protein-induced production of Insulin-like peptides. Recent work indicates a positive correlation between proliferation rates and FSC lifespan in well-fed flies, suggesting that highly proliferative cells have an advantage for niche retention. Paradoxically, we find that constitutive Hh signaling drives rapid FSC loss and premature sterility despite increased proliferation, indicating that additional mechanisms contribute to stem cell lifetime. Here, we demonstrate that constitutive Hh promotes dual induction of autophagy and proliferation in young FSCs, driving their loss from the niche and shortening reproductive lifespan. A similar Hh-dependent mechanism is observed in old, wild-type FSCs, suggesting that age-dependent shifting of the proliferation-autophagy balance promotes reproductive arrest. Strikingly, we find that this balance is exquisitely controlled by coordinated Hh and Insulin signaling to maintain young FSCs in a self-renewing state, with Insulin signaling inhibiting Hh-induced autophagy but not proliferation. Thus, Hh and Insulin control the balance between autophagy and proliferation to ensure FSC fitness during aging, providing a molecular mechanism to connect a balanced diet with healthy egg production.

3 Dietary lipid dependent regulation of the intestinal stem cell lineage by DHR96 and Notch *Rebecca Obniski*, Matthew Sieber, Allan C. Spradling. Department of Embryology, Carnegie Institution for Science, Baltimore, MD.

The Drosophila midgut must optimize the digestion, absorption, and trafficking of dietary nutrients, adapting to utilize available resources in varied environments. For this purpose, the tissue is subdivided into at least ten subregions with specialized functions (1,2). Interestingly, these regions are not established until an adult fly's first

meal, suggesting that signals relayed to the intestine from the environment may play a fundamental role in the final stages of midgut development. However, it remains unclear how individual nutrients may influence cell fate decisions during the differentiation of the intestine. We discovered that by modulating the levels of dietary lipids the ratio of enteroendocrine cells (EEs) to enterocytes (ECs) increased proportionally with the levels of cholesterol in the diet, specifically in lipid absorptive regions of the intestine. Cholesterol-mediated control of EE abundance required the nuclear hormone receptor, DHR96. Previous studies have identified cholesterol as the ligand for DHR96, and characterized it as a master regulator of cholesterol uptake and homeostasis (3). DHR96 gene dosage affected the number of EEs throughout the intestine over a fourfold range. Furthermore, in lipid absorptive regions, DHR96 and dietary cholesterol act synergistically to increase EEs. We show that DHR96 and cholesterol modulate intestinal cell differentiation through effects on Notch signaling. Increasing either DHR96 protein, or dietary cholesterol represses Delta levels in the stem cell, biasing daughters to differentiate along the EE pathway. Overall, our studies have shown that the cellular composition in the intestinal epithelia is in part dictated by the influx of dietary lipids. Moreover, DHR96 functions as a nutrient sensor that monitors cholesterol levels and acts through Notch signaling to couple cues from the diet to the differentiation of the intestinal stem cell lineage.

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4 Deciphering the biological role and molecular mechanism of germline de-differentiation in flies *Salvador Herrera*[1], Erika Bach[1,2]. 1) Department of Biochemistry and Molecular Pharmacology. NYU School of Medicine, New York; 2) Helen L. and Martin S. Kimmel Center for Stem Cell Biology. NYU School of Medicine New York.

Exhaustion of stem cells and their niche is a hallmark of aging. The testis of *Drosophila melanogaster* offers an excellent model to study this process, with well characterized signaling pathways that control stem cell maintenance. Additionally, the *Drosophila* testis provides the opportunity to study de-differentiation, whereby differentiating spermatogonia de-differentiate into new germline stem cells (GSCs). Previous work has revealed that during aging the proportion of de-differentiated GSCs increases in the GSC pool (Cheng, *Nature* 2008), suggesting that this process may counteract the decline of stem cells normally observed in aged males. In order to test this hypothesis, we blocked de-differentiation by overexpressing the differentiation factor *bag of marbles* (*bam*) in already differentiating spermatogonia. By simultaneously labeling the lineage of these cells, we monitored their contribution to the GSC pool. Strikingly, we have discovered that blocking de-differentiation has no impact on the preservation of the GSC pool, ruling out a role for de-differentiation in normal aging conditions under standard laboratory conditions. However, we found that under challenging conditions like starvation, which decreases the GSC pool, de-differentiation provokes a faster recovery of stem cell number. In starved flies, the GSC pool takes 3 days to recover to pre-starvation levels, but when de-differentiation is blocked, this recovery takes significantly longer - 7 days. Additionally, we observed that this delay correlates with decreased male fertility. Moreover, after several rounds of starvation and re-feeding (which we argue may mimic aging conditions in the wild), de-differentiation-compromised testes show a pronounced decay both in the number of GSCs and their ability to differentiate. Overall, we think that de-differentiation provides a mechanism to regenerate the germline and ensure male fertility under stressful conditions. We are also interested in describing the molecular mechanism that reverts these differentiating cells to stem cells. Our data show that the JNK signaling pathway activity is autonomously required in the germline for de-differentiation. Intriguingly, this molecular mechanism resembles what it is known about regeneration processes in *Drosophila* epithelial tissues.

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5 Tricellular junctions regulate intestinal stem cell behavior to maintain homeostasis *Martin Resnik-Docampo*[1], Christopher Koehler[1], Rebecca Clark[2,5], Joseph Schinaman[2], Vivien Sauer[1], Daniel Wong[1], Sophia Lewis[1], Cecilia D'Alterio[1], David Walker[2,3], Leanne Jones[1,3,4]. 1) Department of Molecular, Cell, and Developmental Biology, UCLA, Los Angeles, CA; 2) Department of Integrative Biology and Physiology, UCLA, Los Angeles, CA; 3) Molecular Biology Institute, UCLA, Los Angeles, CA; 4) Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA; 5) School of Biological and Biomedical Sciences, Durham University, 24 Durham DH1 3LE, UK.

Aging results in loss of tissue homeostasis across taxa. In the intestine of *Drosophila melanogaster*, advanced age is correlated with an increase in intestinal stem cell (ISC) proliferation, a block in terminal differentiation of progenitor cells, activation of inflammatory pathways, and increased intestinal permeability. However, the causal relationships between these phenotypes remain unclear. Here, we demonstrate that aging results in altered localization and expression of Septate Junction (SJ) proteins in the posterior midgut, which is quite pronounced in differentiated enterocytes (ECs) at tricellular junctions (TCJ). Acute loss of the TCJ protein Gliotactin (Gli) in ECs resulted in increased ISC proliferation and a block in differentiation in intestines from young flies, demonstrating that

compromised TCJ function is sufficient to alter ISC behavior in a non-autonomous manner. Blocking the Jun N-terminal kinase (JNK) signaling pathway was sufficient to suppress changes in ISC behavior, but had no effect on loss of intestinal barrier function, as a consequence of Gli depletion. Furthermore, loss of Gli in ISCs leads to a block in differentiation of progenitor cells, suggesting that expression of SJ proteins and assembly of SJ are fundamental steps in terminal differentiation. Our work demonstrates a pivotal link between TCJ, stem cell behavior, and intestinal homeostasis and provides new insights into causes of age-onset and gastrointestinal diseases.

6 Grainyhead regulates midgut stem cell function *Gary Hime*, Nicole Dominado. Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia.

Snail family transcription factors are required for maintenance of intestinal epithelial stem cell pools and for regulation of daughter cell lineage choice in both mammals and *Drosophila*. This family of proteins and other mesenchymal inducers have been shown to be associated with stem cell potential in a variety of epithelial tissues but it remains curious that these stem cells maintain an epithelial identity. We considered that other proteins may also be required to maintain intestinal epithelial stem cell (ISC) identity in the presence of Snail proteins. We have identified that loss of Grainyhead results in loss of ISCs and a reduction in the size of clones that originate from ISCs. The *grh* gene produces 7 transcripts that generate two forms of protein, GRH.O and GRH.N, dependent upon splicing of exon 5. GRH.O has previously been suggested to be specific to neural tissue but we have shown that GRH.O is required for maintenance of ISCs in the posterior midgut. Ectopic expression of GRH.O results in an expansion of esg+ve cells (ISCs and enteroblasts) however expression of GRH.N results in loss of ISCs. We suggest that GRH.O and GRH.N act in opposing manners to regulate ISC numbers. Levels of *grh* expression are also crucial to correct regulation of ISC biology as *grh* transcripts are found at very low levels in the midgut but GRH is transiently upregulated during regeneration of injured midguts.

7 The Tip60 complex interacts with Myc in *Drosophila* neural stem cell maintenance and polarity *Katja Rust*[1,2], Manu Tiwari[1,3], Andreas Wodarz[1,3]. 1) Anatomy I / Molecular Cell Biology, University Medical Center, Cologne, Germany, Germany; 2) IMPRS Program Molecular Biology, Goettingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences (GGNB); 3) Cologne Cluster of Excellence (CECAD) in Cellular Stress Responses in Aging-associated Diseases.

The *Drosophila* nervous system originates from repeated asymmetric neural stem cell divisions. Protein complexes regulating neural stem cell polarity are highly conserved in mammals. As misregulation of polarity may result in overproliferation or loss of the stem cell, regulators of polarity are extensively studied.

We have identified the Tip60 chromatin remodeling complex as a key factor for the maintenance of larval neural stem cells. Hereby, the Tip60 complex interacts with the transcription factor Myc to regulate gene expression. Our data indicate that Myc might recruit the Tip60 complex to target gene promoters to incorporate the histone variant H2Av and acetylate H4 to control transcription. By RNA-sequencing we unraveled that the Tip60/Myc network represses genes for neural differentiation and induces genes for self-renewal.

Loss of Tip60 components or Myc results in premature differentiation and thus stem cell loss. Neural stem cells divide slower, display reduced cellular growth and nuclear entry of Prospero, a transcription factor promoting differentiation, and finally differentiate terminally.

Remarkably, the Tip60/Myc network is further required for neural stem cell polarity and asymmetric division by activation of aPKC expression. aPKC acts as a protein kinase in the PAR complex, a key regulator of cell polarity, and supports neural stem cell self-renewal. Therefore, the Tip60/Myc network maintains neural stem cells by inducing key factors of neural stem cell identity.

Together, like mammalian pluripotent stem cells, *Drosophila* neural stem cells depend on the Tip60/Myc transcriptional network. The Tip60/Myc network acts on multiple levels by repressing differentiation, activating self-renewal and enabling asymmetric stem cell division.

8 4D dynamics of cell division, differentiation, and loss during midgut renewal in live adult Drosophila *Judy Martin*, Erin Nicole Sanders, Paola Moreno-Roman, Shruthi Balachandra, Leslie Jamarillo Koyama, Lucy Erin O'Brien. Molecular & Cellular Physiology, Stanford University, Stanford, CA.

Comprehensive understanding of adult organ renewal requires knowledge of stem and differentiated cell behaviors within three-dimensional tissues over time. However, such information has been difficult to obtain *in vivo* because of challenges in observing internal organs and interpreting complex, spatiotemporal data. To overcome these challenges, we perform long-term, wide-field, continuous imaging of the midgut at high cellular resolution within live animals and use cell-specific markers to quantitatively analyze cell positions and fates. We find that stem cells display highly variable division orientations, teetering throughout a single mitosis, but lock into a linear orientation when 'sandwiched' between two enteroblast progenitors. Using a GFP reporter for Notch activity, we observe that cells can delay fate commitment for up to 12 hours after birth. Notch activation intensifies over several hours and is marked by initial but not sustained contact with Delta-expressing stem cells. Terminal enterocytes are shed through progressive

constriction of the apical surface, which causes rosette formation and, ultimately, lumenal extrusion. These kinetics of midgut cell division, differentiation, and loss form a basis for dynamic understanding of epithelial tissue renewal.

9 Sexually dimorphic action selection of sex and sleep in *Drosophila* *D Sitaraman*[1], D Chen[2], N Chen[3], X Jin[4], C Han[2], B Baker[3], M Nitabach[4], Y Pan[2]. 1) Behavioral Neuroscience, Dept. of Psychological Sciences, University of San Diego, San Diego, CA; 2) The Key Laboratory of Developmental Genes and Human Disease, Institute of Life Sciences, the Collaborative Innovation Center for Brain Science, Southeast University, Nanjing, Jiangsu, China; 3) Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA, USA; 4) Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA.

Animals prioritize the execution of behavioral options based on environmental cues and internal states. However, the neural mechanisms underlying such action selection decisions remain poorly understood. Here we studied how two fundamental behaviors, sex and sleep, are prioritized in *Drosophila*. We found that an increased need for sleep inhibits male sexual behavior by decreasing the activity of the male-specific P1 neurons that co-express the sex determination genes fruM and dsx, but does not affect female sexual behavior. Further, we have delineated a sex-specific action selection circuit wherein the P1 neurons encoding increased courtship drive suppress male sleep by activating fruM-positive sleep-controlling circadian clock DN1 neurons. In addition, we found that fruM and dsx differentially control sexually dimorphic sleep patterns. These studies reveal that action selection for sleep and sexual behaviors is sexually dimorphic, and reciprocal inhibition between sex and sleep circuitries is responsible for action selection of these behaviors.

10 The gustatory basis of protein homeostasis. *Samuel J. Walker*, Kathrin Steck, Pavel Itskov, Celia Baltazar, Carlos Ribeiro. Champalimaud Research, Champalimaud Centre for the Unknown, Lisboa, Portugal.

Animals must adapt their behaviour to multiple ongoing internal states. In Drosophila, nutrient-specific hungers such as protein and salt appetite are driven by metabolic and reproductive states, but the neuronal mechanisms processing and integrating these states are unknown. Furthermore, despite its status as a key component of the fly's diet, the sensory neurons that mediate feeding on yeast are unknown. Here, we employ a novel technology, the flyPAD, to dissect distinct aspects of feeding behaviour, and a chemically defined diet to differentiate the effects of different dietary components on feeding microstructure. Using these tools, we show that different internal states affect specific aspects of feeding on yeast, flies' main source of dietary protein, to support protein homeostasis. Furthermore, we identify a distinct class of gustatory receptor neurons (GRNs) that are necessary for yeast feeding, and show that GRNs within this class in different anatomical locations mediate these different components of yeast feeding behaviour. Specifically, while GRNs on the labellum appear to be important for the initiation of feeding bursts, specific GRNs in the pharynx are necessary for sustaining yeast feeding bursts. We further identify a class of second-order gustatory neurons that regulate the sustaining of yeast feeding bursts according to the fly's metabolic state.

To characterise the response properties of GRNs, we employ 2-photon calcium imaging, and show that specific labellar GRNs respond selectively to yeast. This response is modulated by the flies' dietary pretreatment: food high in yeast reduces the gain of taste peg GRNs, and this response correlates with changes in the probability of feeding burst initiation. Furthermore, female reproductive state is a key modulator of protein appetite. While mating strongly affects feeding burst initiation, it has no effect on the response of yeast GRNs, indicating that this internal state acts on higher-level gustatory circuits. The emerging picture is that specific yeast-sensing neurons mediate distinct aspects of feeding microstructure on a single substrate, but act redundantly to support total intake; and, further, that distinct internal states act at different levels of sensory processing to shape nutrient-specific hungers.

11 Here come zombie flies: *Entomophthora muscae*, a model behavior-manipulating pathogen of *Drosophila melanogaster* *Carolyn Elya*[1], Michael Bronski[1], Michael Eisen[1,2,3]. 1) Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Integrative Biology, UC Berkeley, Berkeley, CA; 3) Howard Hughes Medical Institute, UC Berkeley, Berkeley, CA.

Many parasites evoke striking behavioral changes in their animal hosts, but how they achieve this is poorly understood at the molecular level. This is due in large part to the difficulty of studying these non-model organisms with limited molecular tools. Recently, we discovered a strain of the behavior-manipulating fly pathogen *Entomophthora muscae* infecting wild fruit flies in Northern California and have developed methods to reliably propagate the infection in lab-reared *Drosophila melanogaster*. Our infected flies manifest the moribund behaviors characteristic of *E. muscae* infections: on their final day of life they climb to a high location (summiting), extend their proboscis and become affixed to the substrate with a fungal-produced adhesive, then raise their wings in a characteristic death pose that clears a path for spores that are forcibly ejected from their abdomen to land on and infect other flies. We are now using the well-developed tools of *D. melanogaster* molecular genetics and neurobiology to discover how the fungus induces this dramatic behavioral manipulation. Using RNAseq to monitor both fungal and host gene expression, we have observed a strong host immune response and a multifaceted *E. muscae* transcriptional program over the course of infection. To make better sense of these data, we have

sequenced our *E. muscae* isolate's genome, which is unusually large for fungi at around 1.3 Gb. We are complementing these genomic studies with detailed behavioral studies in wild-type and mutant flies, that reveal, for example, complex interactions between the fungus and the fly circadian system, as well as external cues, in controlling the timing of death and its associated behaviors.

12 Clean up your action selection: How the brain organizes motor sequences in fly grooming *Julie Simpson*, Primoz Ravbar, Shingo Yoshikawa, Sunanda Marella, Neil Zhang, Li Guo. MCDB, UCSB, Santa Barbara, CA.

Animals can solve the problem of choosing between competing actions by executing behaviors in sequence. Fly grooming provides an opportunity to identify the neural circuits that compare sensory inputs and arbitrate among motor outputs to coordinate a flexible cleaning progression. Grooming is evoked by dust on the body surface and consists of individual cleaning movements targeted to body parts: head and front leg rubbing constitute an anterior motif, while abdominal, wing, or thoracic sweeps alternate with back leg rubbing make up posterior motifs. We use automated analysis of grooming movements to show that the brain affects the balance of grooming motifs and the anterior-to-posterior progression. The activation of mechanosensory bristle neurons evokes cleaning movements, but addition of dust increases time spent grooming, suggesting some contribution from other sensory modalities. Our screens for specific neuronal types that affect grooming reveal some that mediate between leg rubbing and body sweeps, and others that increase the probability of posterior motifs. We continue to search for neurons that control the flexibility and progression of this innate motor sequence using behavior quantification, optogenetic manipulation of neural activity, and functional imaging in candidate neurons.

13 Encoding of Larval Body Movements and Position by Directionally Selective On/Off Proprioceptive Neurons *W.D. Tracey*[1], E. Heckscher[2], M. Krieg[3]. 1) Department of Biology, Indiana University Bloomington; 2) University of Chicago; 3) Stanford University.

The nervous system of the *Drosophila* larva produces rhythmic patterns of motor neuron activity that drive larval crawling. In the absence of sensory input from proprioceptive sensory neurons, waves of larval muscle contraction still occur, but they are highly uncoordinated and slow, suggesting that sensory feedback is necessary for proper functional locomotion. It has been proposed that the Class I multidendritic neurons (dda-E, dda-D and vp-da) and the bipolar multidendritic (i.e. dbd) neurons together send a "mission accomplished" signal to the larval brain when a body segment is fully contracted. Here, we have used high-speed confocal microscopy in moving and intact animals to investigate the pattern of dendrite deformations that occur in proprioceptive dendrites during larval locomotion. Interestingly, we observe that distinct patterns are seen during forward and reverse locomotion. During forward locomotion, the dendrites of dda-D remain relatively undisturbed, while the dendrites of dda-E bend inward. The opposite pattern is seen during backwards locomotion. Thus, backwards and forward locomotion produce mirror image patterns of dendrite deformation. The dendrites of the d-bd neuron are under tension when the segment is relaxed and are seen to buckle during a muscle contraction. To investigate how these patterns of deformation reflect neuronal activity we observed GCaMP6.0F in our preparation and recorded movement related signals in five peripheral sensory neurons (dda-E, dda-D, dmd1, dbd, and vp-da). Unique calcium responses were seen in each of the different neurons. The dda-E and vp-da neurons showed "ON" responses that were strongest during anteriorly progressing waves of muscle contraction (ie. crawling forwards) while dda-D showed ON responses that were strongest during backwards locomotion. The dmd-1 neuron showed on responses during both forward and backwards crawling. Dendrites of the md-bp neuron showed high calcium signals while stretched (during segmental relaxation) and an "OFF" response during segmental muscle contractions. Surprisingly, our findings indicate that the proprioceptive inputs of forward and reverse locomotion are mirror images of each other. The neural signals of backwards locomotion are not equal to the signals of forward locomotion if they are played in reverse. Furthermore, unique signals from 5 neurons provide complex information to the larval brain that is beyond what is necessary to send a simple "mission accomplished signal." We propose that this information is sufficient to provide the larval brain with a precise computation of body position.

14 Gut microbial modification of *Drosophila* locomotor behavior *Catherine Schretter*[1], Jost Vielmetter[2], Imre Bartos[3], Zsuzsa Marka[3], Szabolcs Marka[3], Sonja Hess[4], Sarkis Mazmanian[1]. 1) Division of Biology and Bioengineering, California Institute of Technology, Pasadena, CA; 2) Protein Expression Center, Beckman Institute, California Institute of Technology, Pasadena, CA; 3) Department of Physics, Columbia University, New York, NY; 4) Proteome Exploration Laboratory, Beckman Institute, California Institute of Technology, Pasadena, CA.

Microbes inhabiting the gastrointestinal tract influence metabolism and immunity in their hosts. However, relatively little is known about how this microbial environment influences the behavior of the host. Due to gut bacterial participation in host energy storage, we hypothesized that the microbiota plays a role in locomotor behavior in *Drosophila melanogaster*.

We found significant differences in the gait, speed, activity, and climbing behavior of adult fruit flies without a

microbiota (axenic) compared to those with a diverse microbiota (conventional). To evaluate if these behavioral differences were due to a general feature of the gut microbiota or specific to particular species, we performed mono-colonization experiments in axenic fruit flies. These experiments revealed that only specific species of *Lactobacilli* and *Acetobacter* influence locomotor behavior. Changes in the levels of these bacteria in mono-colonized and conventional fruit flies also significantly correlated with their changes in speed. Utilizing enzymatic and proteomic strategies, we have uncovered a small group of 30-70kDa bacterial-derived proteins that can modulate this host behavior. While their effects on behavior were found to be independent of the immune deficiency (IMD) pathway as well as antimicrobial peptide expression, these bacterial-derived proteins did decrease levels of hemolymph sugars. Furthermore, we found that the behavioral effects of these proteins are mediated through octopamine. Our results provide evidence that gut bacteria and their products can act as endogenous stimuli that are integrated into host behavioral responses through a conserved energy homeostasis pathway.

15 Complex aminergic regulation of the *Drosophila* egg-laying circuit *Sonali Deshpande*[1], Daniel Suto[1], James Asuncion[1,2], Pei-Tseng Lee[3], Hugo Bellen[3,4,5,6,7], David Krantz[1]. 1) Department of Psychiatry, University of California, Los Angeles, Los Angeles, CA; 2) Medical Scientist Training Program, University of California, Los Angeles, CA; 3) Department of Molecular and Human genetics, Baylor College of Medicine, Houston, TX; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 5) Howard Hughes Medical Institute; 6) Program in Developmental Biology; 7) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, United States.

The biogenic amines dopamine, serotonin and octopamine regulate multiple behaviors in *Drosophila,* but the underlying mechanisms and interactions with other signaling pathways remain unclear. To investigate these questions, we have applied new techniques to the egg-laying (oviposition) circuit. Classic studies of oviposition in larger insects suggest that glutamate causes contraction of the oviduct, whereas octopamine promotes relaxation. The opposing effects of glutamatergic and octopaminergic pathways suggest that descending pathways could differentially activate each cell type. To test this possibility, and more precisely determine the mechanism by which glutamatergic and octopaminergic components of this circuit interact, we have developed a preparation for live imaging of both neuronal firing in the thoracic ganglia, and muscle contractions in the oviduct. We find that exogenous application of glutamate activates octopaminergic but not glutamatergic neurons in the thoracic ganglion, suggesting a possible mechanism for differential activation of each pathway. We observe activation of at least two different firing patterns in octopaminergic neurons, suggesting an additional level of complexity. In a second set of experiments, we find that exogenous application of octopamine differentially activates two specific subdomains of the oviduct musculature. Further experiments using newly developed reporter lines will be used to determine different receptors drive the response of each subdomain. Our data provides new information about the unexpected complex mechanisms underlying an important model of aminergic neuromodulation.

16 *"not my type":* a candidate gene for behavioral isolation in *Drosophila* *Tabashir Chowdhury*, Amanda Moehring. Biology, University of Western Ontario, London, Ontario, Canada.

As one of the major tenets of speciation, reproductive isolation maintains species boundaries by preventing gene flow. Among the numerous reproductive isolating mechanisms, behavioral isolation is one of the earliest acting barriers. This mechanism involves discordance in mating strategies between diverged taxa, with females exhibiting a preference for conspecific male traits. *Drosophila* is ideally suited for studying the genetic mechanisms underlying complex behaviors due to its stereotypic multimodal courtship ritual, and a comprehensive genetic toolkit. Although several candidate genes have been linked to behavioral isolation in *Drosophila*, the genetic basis for species-specific female preference remains unclear. Using complementation testing and mating assays I have confirmed a candidate behavioral isolation gene for species-specific female preference: *not my type (nmt)*. This gene plays a significant role in species-specific female preference for conspecific males. CRISPR-generated deletions of exons of *nmt* have further narrowed down species-specific female preference to a particular set of exons. This sets the foundation for understanding the mechanisms by which this gene affects female preference and influences behavioral isolation across *Drosophila* species.

17 Investigating molecular mechanisms of microcephaly through mitotic spindle-independent pathways *T. Schoborg*, L. Smith, C. Fagerstrom, N.M. Rusan. Cell Biology & Physiology Center, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by reduced brain size and life span. While the clinical aspects of the disorder are well characterized, the molecular mechanism remains poorly understood. The currently accepted hypothesis favors cell division defects induced by mitotic spindle errors as the cause of the disorder, as mistakes in chromosome segregation can lead to abnormal differentiation and apoptosis. Either of these scenarios can reduce neuron/glia numbers, which in turn results in a smaller brain. The most commonly mutated gene in human MCPH patients, *Abnormal Spindle-Like, Microcephaly Associated (ASPM)* is

known to be important for proper centrosome and mitotic spindle function during mitosis. However, our recent analysis of the *Drosophila melanogaster* ortholog, *Abnormal Spindle (Asp),* showed that mitotic spindle & cell division defects are not the primary cause of MCPH in *Asp* mutant animals, suggesting the current model needs to be revised. To do so, we are establishing a set of criteria that defines MCPH using novel imaging methods such as microcomputed tomography (micro-CT) and optical sectioning of intact adult heads and brains, coupled with sophisticated image segmentation and registration algorithms. We are also performing single neuron labeling in adult brains to identify affected populations of neurons/glia in *Asp* mutant animals. Our data has revealed that a null mutation of *Asp* disrupts proper development of the adult optic lobes, including a severe disorganization of the lobula complex (LOX) neuropils. Central brain neuropils retain their morphology in the *Asp* mutant, similar to wild-type animals, suggesting MCPH is restricted to specific areas of the brain in *asp* mutants. Our structure-function analysis of the protein shows that a 573 amino acid region of Asp's N-terminus is sufficient to rescue the LOX complex defects in the *Asp* null mutant. We are currently investigating multiple pathways through which this N-terminal fragment suppresses MCPH, including a regulatory role in the interphase nucleus and a role in radial migration of optic lobe neurons during metamorphosis.

18 The Polyadenosine RNA Binding Protein, Nab2, is a Functional Ortholog of the Human Intellectual Disability Gene ZC3H14 and Regulates Mushroom Body Development *Seth Kelly*[1], Michael Kahl[1], Ginelle Doerr[1], Rick Bienkowski[2], Kenneth Moberg[2]. 1) Department of Biology and Neuroscience Program, The College of Wooster, Wooster, OH; 2) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA.

Intellectual disability (ID) is a broad collection of debilitating diseases that significantly affect cognitive function in human patients. Despite the prevalence of genetically heritable forms of ID and the recent increase in the identification of human genes linked to ID, the molecular function of the proteins encoded by many of these genes is unknown. Several recent studies have identified common cellular pathways disrupted in intellectually disabled patients. However, these analyses still underscore the importance of understanding the function of newly identified ID genes so they can be linked to one of these common pathways. As a case in point, disruption of the human *ZC3H14* gene has been linked to human ID, but very little is known about the cellular function of the ZC3H14 protein. Patients containing two null alleles of *ZC3H14* present with moderate to severe ID, but lack other dysmorphic features commonly comorbid with ID, suggesting that *ZC3H14* may play a principle role in the nervous system. In order to better characterize the function of ZC3H14 in nervous system function and development, we developed a model of ZC3H14 loss using *Drosophila* lacking the *ZC3H14* ortholog, called Nab2. Our studies have demonstrated that ZC3H14 and Nab2 both encode CCCH-zinc finger containing polyadenosine RNA binding proteins that control poly(A) tail length. Loss of Nab2 also causes significant memory deficits using a courtship suppression learning and memory assay. Finally, Nab2 null flies have profound axonal guidance defects in the mushroom bodies (MBs), a well-characterized brain region required for learning and memory in the fly. Interestingly, we have demonstrated that loss of Nab2 causes changes in the level of *rutabaga* and *dunce* mRNA transcripts, suggesting that Nab2 mutants may have alterations in steady-state cyclic AMP (cAMP) levels. These changes in cAMP levels as well as alterations in the activity of downstream effectors could underlie the axon guidance and learning and memory defects observed in Nab2 null flies. Together this data suggests that Nab2 and ZC3H14 may bind to and post-transcriptionally regulate the expression of specific transcripts important for regulating axonal pathfinding during neuronal development.

19 MARRVEL: Integration of public resources to prioritize human genetic variants for study in model organisms R. Al-Ouran[1,11], *J. Wang*[2,3,11], Y. Hu[4,11], Y.W. Wan[1,5,6], M. Wangler[1,6,7,8], S. Yamamoto[1,8], H.T. Chao[1], A. Comjean[4], S.Y. Kim[1], K. Shefchek[9], S. Mohr[4], N. Perrimon[4,10], H. Bellen[1,2,8,10], Z. Liu[1,6]. 1) Jan and Dan Duncan Neurological Research Institute, Houston, TX 77030, USA; 2) Program in Developmental Biology, Baylor College of Medicine (BCM), Houston, TX 77030; 3) Medical Scientist Training Program, BCM, Houston, TX 77030; 4) Drosophila RNAi Screening Center, Dept of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA; 5) Dept of Obstetrics and Gynecology, BCM, Houston, TX 77030; 6) Dept of Pediatrics, BCM, Houston, TX 77030; 7) Texas Children's Hospital, Houston, TX 77030; 8) Dept of Molecular and Human Genetics, BCM, Houston, TX 77030; 9) Dept of Medical Informatics and Epidemiology, and Oregon Health and Science University Library, Oregon Health and Science University, Portland, Oregon 97239; 10) Howard Hughes Medical Institute, Houston, TX 77030; 11) Contributed equally.

As whole exome and genome sequencing are incorporated into personal health care, we are faced with an abundance of rare variants and a great need for *in vivo* functional studies. To understand the impact of variants and to provide a genetic diagnosis, it is critical to rapidly integrate all available knowledge from human genetic data sets as well as molecular and biological data from model organisms for genes and variants of interest. We sought to streamline human variant prioritization for study in model organisms through development of MARRVEL (Model organism Aggregated Resources for Rare Variant ExpLoration, http://marrvel.org/). MARRVEL makes it possible to examine conservation of a specific gene and variant across model organisms and displays a concise summary of

what is known about orthologs. To automate the searches and provide a unified view of data for a given query variant and gene, MARRVEL first extracts data from public human data bases such as OMIM, ExAC, Geno2MP, DGV, and DECIPHER. Next, the protein sequences of orthologs found in any of nine organisms (budding yeast, fission yeast, worm, fly, zebrafish, frog, rat, mouse, and human) are aligned and the protein domains displayed through integration with DIOPT. Key biological and genetic features including tissue expression pattern and protein interactions are then extracted from databases such as SGD, WormBase, FlyBase, ZFIN, and MGI. Altogether, MARRVEL provides an efficient online tool to select the appropriate model organism in which to study candidate genes and variants, and sets the stage for diagnostic interpretation and in-depth studies of novel pathogenic mechanisms.

20 Nrf2 and Epigenetic Aging *Dirk Bohmann*[1], Yang Cheng[1], Min Tian[1], Hoff Robert[1], Pitoniak Andrew[1,2]. 1) Biomedical Genetics, University of Rochester, Rochester , NY; 2) Science, Technology, Engineering, & Mathematics, Jamestown Community College, Jamestown, NY.

The Drosophila cap'n'collar locus encodes leucine zipper transcriptions factor with homology to mammalian Nrf1 and Nrf2. The CncC protein activates gene expression programs that defend flies against xenobiotic and oxidative insults, as well as unfolded protein stress. Consistent with these protective functions, CncC can ameliorate pathologies that are caused or exacerbated by oxidative or unfolded proteins. We and others have demonstrated this effect in fly models for Parkinson's and Alzheimer's disease. Gain of CncC function can also extend lifespan, implicating the transcription factor in the control of aging. We have shown that the capacity of flies to activate the expression of CncC target genes in response to stress exposure declines with increasing age of the organism. Chromatin analyses by DNAse hypersensitive site mapping indicate that this phenotype is caused by an age-associated loss of epigenetic organization around the CncC target gene promoters. With increasing age of the organism, these transcription control regions lose their youthful open chromatin conformation and are no longer accessible for binding by activated CncC transcription factors. We have developed genetic and pharmacological strategies that prevent this epigenetic degeneration to preserve signal dependent transcriptional responses in old animals. This preservation of transcriptional competence results in increased fitness in later stages of life and extended lifespan. Our studies suggest that epigenetic degeneration is a fundamental cause of aging, Based on these findings, we present possible strategies to slow down epigenetic aging and to extend healthspan and lifespan.

21 *SLP-2* interacts with PINK1 in regulating mitochondrial function and bioenergetics in a *Drosophila* Parkinson's disease model *Sreehari Kalvakuri*[1], Andrew A Hicks[2], Irene Pichler[2], Rolf Bodmer[1]. 1) Development and Aging program, SBP Medical Discovery Institute, La Jolla, CA; 2) 2Center for Biomedicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy - Affiliated institute of the University of Lübeck, Germany.

Parkinson's disease (PD) is one of the two most common neurodegenerative disorders. Among the various model organisms, the *Drosophila* has emerged as an efficient system to study PD genes and to screen for genetic modifiers of PD pathology. Mutations in *Drosophila* homologues of PD genes result in phenotypes that are deemed remarkably equivalent to those observed in PD patients, including motor dysfunction, malfunctioning of dopaminergic (DA) neurons, reduced dopamine levels and mitochondrial dysfunction. Our primary goal is to find novel molecular targets that would ameliorate PD pathology. Towards this, we have identified Stomatin Like Protein-2 (*SLP-2)* in a genetic screen for novel genetic modifiers of mitochondrial dysfunction caused by *PINK1* deficiency. Knockdown of *SLP-2* significantly enhanced *PINK1* dependent phenotypes, whereas overexpression of *SLP-2* completely suppressed *PINK1*-induced phenotypes in indirect flight muscles and in the DA neurons of *PINK1* mutants. We also find that *SLP-2* mediated rescue of *PINK1* mutants is dependent on gene products that regulate cardiolipin biosynthesis. Knockdown of cardiolipin synthase (CLS) and/or Tafazzin (TAZ) abrogates the rescue by *SLP-2* overexpression. Our data indicate that *SLP-2* regulates mitochondrial bioenergetics and mitophagy probably by interacting with cardiolipin-enriched microdomains on the inner membrane of mitochondria and thus assisting efficient assembly and maintenances of OXPHOS super complexes, which will reduce ROS production and mitochondrial damage. Interestingly, in contrast to *PINK1* overexpression, activation of *SLP-2* also suppressed *parkin*-dependent PD phenotypes in indirect flight muscles and DA neurons, which suggests *SLP-2* acts in parallel or downstream of *parkin*. Thus, our data demonstrate a novel molecular pathway that works in downstream or in parallel to *PINK1/parkin*, providing a novel therapeutic target to potentially treat PD patients.

22 Microenvironmental autophagy promotes tumor growth *N.S. Katheder*[1,2], R. Khezri[1,2], F. O'Farrell[1,2], S.W. Schultz[1,2], A. Jain[1,2], M.M. Rahman[1,2], K.O. Schink[1,2], T. Theodossiou[3], T. Johansen[4], G. Juhász[5,6], D. Bilder[7], A. Brech[1,2], H. Stenmark[1,2], T.E. Rusten[1,2]. 1) Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, N-0379 Oslo, Norway; 2) Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Montebello, N-0379 Oslo, Norway; 3) Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, N-0379 Oslo, Norway; 4) Molecular Cancer Research Group, Institute of Medical Biology, UiT - The Arctic University of Norway, 9037 Tromsø, Norway; 5) Institute of Genetics,

Biological Research Centre, Hungarian Academy of Sciences, Szeged, H-6726 Hungary; 6) Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest H-1117, Hungary; 7) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720-3200, USA.

Malignant tumors develop in intimate interaction with the microenvironment and can activate autophagy, a catabolic process which provides nutrients during starvation. How autophagy is regulated by tumors *in vivo* and if it impacts tumor growth is controversial. Here we demonstrate, using a well-characterized *Drosophila* malignant tumor model, that non-cell-autonomous autophagy is induced in the tumor microenvironment as well as systemically in distant tissues. Tumor growth can be pharmacologically restrained using autophagy inhibitors, and early-stage tumor growth and invasion are genetically dependent on autophagy within the local tumor microenvironment. Induction of autophagy is mediated by *Drosophila* Tumor Necrosis Factor-a and Interleukin-6-like signaling from metabolically stressed tumor cells, while tumor growth depends on active amino acid transport. Importantly, dormant growth-impaired tumors that stem from autophagy-deficient animals reactivate tumorous growth when transplanted into autophagy-proficient hosts. We conclude that transformed cells engage surrounding normal cells as an active and essential microenvironmental contributor to early tumor growth through nutrient-generating autophagy.

23 Systematic analysis of miRNAs in epithelial tumors reveals tumor enhancing and repressing miRNAs *Zhiqiang Shu*, Yi-Chun Huang, William Palmer, Yoichiro Tamori, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

microRNAs (miRNAs) have emerged as an important class of noncoding RNAs that is involved in cancer cell transformation, invasion, and migration; however, the different roles of miRNAs in tumorigenesis are still unclear. To gain insights into how miRNAs influence tumorigenesis, we conducted a small RNA sequencing (RNA-seq) analysis of lgl-RNAi/Ras^{V12} induced epithelial tumors in the *Drosophila* wing disc, and identified miRNAs that had at least two-fold changes in expression levels between the normal and tumor discs. Employing two tumor-sensitized fly strains, we identified 13 onco-miRNAs and 22 potential tumor-suppresor miRNAs that were able to affect lgl-RNAi-induced tumorigenesis. From this study, we also identified 96 passenger miRNAs that had no obvious role in enhancing or alleviating tumorigenesis despite of their changed expression levels. Further studies showed that many of these potential onco-miRNAs and tumor-suppressor miRNAs modulate the activity of signaling pathways such as EGFR, NF-kB, Notch, JAK/STAT, and JNK that contribute to lgl-RNAi induced epithelial tumors.

24 Microbially-mediated ethanol sensitivity: A model system using *Drosophila*, its microbiome, and ingested ethanol *James Angus Chandler*[1], Daniel Rosenthal[1], Michael Eisen[1,2], William Ludington[1]. 1) Molecular and Cell Biology, University of California, Berkeley; 2) Howard Hughes Medical Institute.

The microbiome, the vast community of microbes associated with an organism, has the capacity to modulate the interaction between an animal host and its environment. In particular, the microbiome may influence an animal's ability to deal with ingested toxins. This can have important consequences for the host's ecological range and evolutionary potential. For example, *Drosophila melanogaster* is able survive much higher ethanol concentrations than its sister species, *Drosophila simulans*. Interestingly, my current work finds that *D. simulans* larvae are less sensitive to ingested alcohol if their microbiome has been experimentally removed. A possible mechanism for this is greater ethanol-induced intestinal deterioration in *D. simulans* compared to *D. melanogaster.* In humans, ethanol increases intestinal permeability, thus allowing the translocation of microbes into the body cavity where they accelerate the progression of alcoholic liver disease. My work finds that following long-term ethanol ingestion, the absence of microbes leads to significantly fewer adult flies exhibiting intestinal deterioration upon death. This indicates that a "leaky gut" may explain ethanol-induced death in *Drosophila* and suggests a conserved physiological response to ingested ethanol between flies and humans. Finally, and in contrast to mammalian systems, I find that ethanol ingestion increases the proportion of the bacterium *Lactobacillus plantarum* in the fly microbiome. In mammals, *L. plantarum* reduces intestinal deterioration and mitigates the negative effects of ethanol. My current work is asking if this shift in microbiome composition benefits flies during ingestion of ethanol. My future steps are to explore the genetic and molecular basis of microbial translocation, intestinal integrity, and organ failure in flies. Combined with imaging of microbial colonization in *Drosophila* organs, this work will create a model system for understanding microbe-mediated alcoholic liver disease in humans and will inform how the microbiome may be shaping the ecology and evolution of *D. simulans* and *D. melanogaster* in their natural habitats.

25 Regulation of adult lipid homeostasis by *Drosophila Estrogen-Related Receptor* *Katherine Beebe*, Michael Horner, Carl Thummel. Human Genetics, University of Utah, Salt Lake City, UT.

Nuclear receptors are a large family of evolutionarily conserved transcription factors that play central roles in development, growth and metabolism. Three paralogs make up the Estrogen-Related Receptor (ERR) family in vertebrates: ERRα, ERRβ, and ERRγ. Although *ERRα* is necessary for lipid homeostasis in mammals, neither the tissue-specific nor mechanistic basis of this phenotype is well understood. Previous work from the Thummel lab has demonstrated that the *Drosophila* member of this family, dERR, establishes a glycolytic metabolic state that supports

larval growth. In contrast, adult *Drosophila* physiology does not involve growth or biomass accumulation, but rather requires efficient oxidative metabolism and ATP production to support the demands of flight and reproduction. We thus engineered a conditional allele of *dERR* to eliminate its function selectively during the adult stage, with the goal of identifying possible new functions for this receptor.

dERR mutant adults display reduced fertility and motility, normal levels of glucose, decreased glycogen, and an almost complete lack of stored triglycerides. Consistent with this, *dERR* mutants are sensitive to starvation. RNA-seq and ChIP-seq based analyses reveal a central role for dERR in regulating metabolism, including glycolysis and lipid metabolism, as well as distinct profiles of *dERR*-regulated transcripts between larval and adult stages. Our current studies are focused on determining the molecular mechanisms and tissue-specific functions by which *dERR* maintains proper lipid stores for adult metabolic homeostasis.

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26 Sex differences in the regulation of triacylglycerol breakdown during starvation Marie-Pierre Gauthier, Nazde Edeer, Lianna Wat, Tiffany Chih, *Elizabeth Rideout*. Dept. Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada.

Sex differences in the length of survival after nutrient deprivation are widely recognized; normally, females survive longer than males. While mating contributes to this enhanced female survival, virgin females still outlive virgin males. The mechanisms underlying these sex differences in survival in unmated flies remain largely unknown. Our analysis of survival in males and females revealed significant sex differences in triacylglycerol (TAG) mobilization after nutrient withdrawal. In males, TAG levels decrease rapidly after starvation. In females, TAG levels are unchanged for approximately 24 hr post-starvation. No sex differences in total protein or carbohydrate levels were observed. One important enzyme known to catalyze TAG mobilization following starvation is highly conserved TAG lipase *brummer* (*bmm*). Although no sex differences in *bmm* mRNA expression were observed in fed animals, induction of *bmm* mRNA levels post-starvation was sexually dimorphic. In males, *bmm* mRNA levels rise 2 hr post-starvation; in females, increased *bmm* expression is significantly delayed. Other studies have shown that the adipokinetic hormone (AKH) pathway plays a central role in the transcriptional induction of *bmm* in response to nutrient withdrawal. We found significant differences in the expression of AKH and the AKH receptor, suggesting a model of sex differences in survival whereby decreased AKH pathway activity in females promotes survival via delayed induction of *bmm* mRNA levels and TAG mobilization.

27 Restoration of Metabolic Rhythms Ameliorates Obesity-Induced Progressive Striated Muscle Dysfunction in Drosophila *Girish Melkani*[1,2], Jesus Villanueva[1], Christopher Livelo[1], Brendon Woodworth[1], Hiep Le[2], Satchidananda Panda[2]. 1) Department of Biology, San Diego State University, San Diego, CA; 2) Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA.

The soaring rate of obesity worldwide is associated with a number of comorbidities, including elevated risk for cardiovascular disease (CVD), insulin resistance in cardiac and skeletal muscle, increased mitochondrial burden, and diabetes. Both genetic factors and lifestyle (e.g., excess or aberrant eating) can disrupt the circadian clock and trigger onset and progression of obesity induced-comorbidities. Interventions that improve molecular circadian and metabolic rhythms are potential entry points for managing cardiovascular and metabolic disorders related to obesity. Taking advantage of the short lifespan and extensive genetic tools available in the *Drosophila melanogaster* (fruit fly) model, we have recently showed that regulating daily feeding/fasting rhythms via time-restricted feeding (TRF) attenuates age-linked cardiac dysfunction (Gill et al. *Science*, 2015). Here, we are using this innovative strategy to mitigate diet and genetic obesity-induced comorbidities by managing the duration of caloric intake alone in a Drosophila model. Our genetic obese mutants showed age-dependent deterioration of cardiac and skeletal muscle performance followed by increase triglycerides level and compromised insulin sensitivity. Mutant obese flies also showed sleep perturbation similar to that in humans. Interestingly, obese flies under TRF showed attenuated obesity-induced cardiac and skeletal muscle senescence, possibly by decreasing lipid accumulation, increased insulin sensitivity and by maintaining metabolic homeostasis due to improved sleep. Furthermore, to understand the molecular basis for TRF's striated muscle health benefits, we have identified novel genes/pathway using transcriptomics and their validation through classical genetics is underway. Based upon our findings, TRF promotes the integration of metabolic pathways with the circadian oscillator, thereby optimizing healthy metabolism by maintaining cardiac and skeletal muscle lipid and minimizing mitochondrial dysfunction, and enhancing insulin sensitivity. Our findings are potentially applicable to human health such as in community-based approaches to improve cardiovascular and other health matrix outcomes linked with slowing or preventing obesity-induced cardiac disorders and other comorbidities.

28 Meep is a novel regulator of insulin signaling and diet-induced diabetes *Matthew T Pereira*, Laura Musselman. Biology, SUNY Binghamton, Binghamton, NY.

High calorie diets are associated with multiple human metabolic disorders including type 2 diabetes (T2D), obesity,

and cardiovascular disease. Using Drosophila melanogaster as a model organism, we take a reverse genetics approach to study the biochemistry of diet-induced metabolic disease as they develop. T2D is characterized by insulin resistance in peripheral tissues, which is associated with a variety of complications including cardiomyopathy, retinopathy, neuropathy, and inflammation. We generate a model of T2D by raising Drosophila on high-sugar diets. Using an RNA-seq- based bioinformatics approach, we identified genes regulated by the insulin receptor InR in fat bodies. This allowed us to identify gene expression most highly associated with insulin signaling in this tissue. CG32335 (meep) is a previously uncharacterized gene that is positively regulated by insulin signaling. Meep is highly conserved and has orthologs in 182 organisms including mice, chimpanzees, and zebrafish. The human ortholog, PTD012, is not well-characterized but has a hydrolase fold and esterase activity. These data led us to hypothesize that Meep regulates fat metabolism. Fat body-specific knockdown of meep reduced size and fat storage while increasing mortality and hyperglycemia on high-sugar diets, consistent with a role in promoting insulin signaling. Meep knockdown flies are also developmentally delayed and far less likely to reach adulthood. In addition, meep was differentially expressed along with genes indicating arole in the antimicrobial response. This research focuses on defining the role of Meep in fat body insulin signaling, metabolic homeostasis, and innate immunity.

29 Cell competition promotes developmental stability through a Dilp8/Lgr3-dependent mechanism Albana Kodra[1], Cora Bergantinos[1], Julien Colombani[2], Ditte Andersen[2], Pierre Leopold[2], *Laura A Johnston*[1]. 1) Dept Gen/Dev, Col Phys/Surg, Columbia Univ, New York, NY; 2) Univ. Cote d'Azur, CNRS, Inserm, iBV, 06100 Nice, France.

Drosophila development is a robust process that yields remarkably reproducible body size and bilaterally symmetric appendages, even in the face of environmental or genetic perturbations. Developmental stability is tightly regulated, as small, random deviations from bilateral symmetry - known as fluctuating asymmetry (FA) - are quite rare. Recent work indicates that developmental stability in flies is regulated by Dilp8, a secreted peptide that signals the growth status of imaginal tissues to coordinate their growth with developmental timing. Dilp8 binds to its receptor Lgr3 on specific neurons in the brain to regulate ecdysone production in the prothoracic gland. Loss of *dilp8* or *lgr3* leads to strong FA in adult wings. Previous work suggested that cell competition, a conserved process that optimizes tissue fitness, is required for the precision of wing size control. We find that mutations in genes required for cell competition also greatly increase wing FA, suggesting a mechanistic link between cell competition and Dilp8. Indeed, Myc-induced cell competition is abrogated in a *dilp8* mutant background. We will present the results of experiments designed to decipher the mechanism linking Dilp8 and cell competition to reduce developmental instability and promote animal fitness.

30 Torso-like interacts with the insulin signalling pathway to regulate growth and developmental timing Michelle A. Henstridge, Lucinda Aulsebrooke, Christen K. Mirth, *Coral G. Warr*. School of Biological Sciences, Monash University, Clayton, VIC, Australia.

Membrane Attack Complex / Perforin-like (MACPF) proteins are best known for roles in mammalian immunity, where they function to disrupt cell membranes by forming oligomeric pores. However, several MACPF proteins instead perform roles in development. The *Drosophila* MACPF protein, Torso-like (Tsl), is well known for its role in activating the Torso (Tor) receptor tyrosine kinase to pattern the embryo termini, and our studies suggest it does so by enabling extracellular accumulation of the Tor ligand Trunk[1]. Tor and Tsl also play roles later in development in growth and developmental timing. Tor functions in the prothoracic gland (PG) as the receptor for prothoracicotropic hormone (PTTH), and *tor* mutants show developmental delay and increased body size[2,3]. While Tsl is also expressed in the PG, and *tsl* mutants show developmental delay, *tor:tsl* double mutants show a much more severe delay than either single mutation alone[4]. This suggests that Tsl can act independently of Tor in growth. In support of this, *tsl* mutants have a smaller body size, not larger as seen with loss of function of PTTH/Tor signalling. In fact the phenotype of *tsl* mutants more closely resembles that of mutations in the insulin signalling pathway. To investigate this we performed metabolic studies and found that *tsl* mutants have increased hemolymph glucose and triglyceride content but unchanged hemolymph trehalose levels, consistent with the known metabolic phenotype of insulin pathway mutants. In addition, *tsl* mutants display reduced nutritional plasticity in a similar manner to *chico* mutants. We have also performed genetic interaction experiments between *tsl* and components of the insulin signalling pathway. Over-expression of PI3K in the prothoracic gland using *phm-Gal4* results in a shorter time to pupariation, and this phenotype is fully suppressed in a *tsl* mutant background. Conversely, the reduced pupariation time caused by InR^{CA} overexpression is not suppressed by loss of Tsl, suggesting that Tsl acts upstream of InR. We therefore tested for interaction with the dILPs. Flies lacking three dILPs (2, 3 and 5) show a greatly extended time to pupariation, and this is not rescued by removal of Tsl. Taken together, our data suggests that Tsl plays a role in activation of the insulin receptor by the dILPs.

1. Johnson, Henstridge *et al.* (2015) *Nat. Commun.* **6**, 8759; 2. Rewitz *et al.* (2009) *Science* **5958**, 1403-1405 ; 3. Grillo *et al.* (2012) *Sci. Rep.* **2**, 762; 4. Johnson *et al.* (2013) *PNAS* **110**, 14688-14692

31 Maintenance of proteostasis by an effector caspase. *S.M. Gorski*[1,2], C. Choutka[1,2], L. DeVorkin[1,2], N.E. Go[1,2], Y-C. Hou[1,2], A. Moradian[1], G.B. Morin[1]. 1) Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada; 2) Dept of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

Proteostasis requires the integrated control of several pathways to maintain cellular integrity in response to stress. The two main degradative pathways that contribute to proteostasis are the ubiquitin-proteasome system (UPS) and autophagy, but how they are molecularly coordinated is not well understood. In this study, we identified an essential role for the effector caspase Dcp-1 in the activation of compensatory autophagy when proteasomal activity is compromised.

We conducted an IP/MS and RNAi screen that led to the identification of candidate regulators of Dcp-1-mediated autophagy. We focused further on one of the identified regulators, Hsp83, due to its human homolog Hsp90 that has links to disease, proteostasis and an ambiguous role in autophagy. *In vivo* studies revealed that loss-of-function *Hsp83* mutants induced autophagy and cell death during *Drosophila* mid-oogenesis. The loss of Hsp83 also led to elevated levels of cleaved and pro-Dcp-1 which were not due to transcriptional regulation. As an explanation for elevated levels of Dcp-1, we investigated the functionality of the UPS, and found that *Hsp83* mutants had decreased proteasomal activity. The levels of pro-Dcp-1 were increased in flies with suppressed proteasomal activity supporting that pro-Dcp-1 itself is regulated by the proteasome. Surprisingly, analyses of *Dcp-1; Hsp83* double mutants showed that the caspase was not required for cell death in this context, but instead was essential for the ensuing compensatory autophagy, female fertility, and organism viability. Our work not only reveals unappreciated roles for Hsp83 in proteasomal activity and regulation of Dcp-1, but also identifies an effector caspase as a key regulatory factor for sustaining adaptation to cell stress *in vivo*.

32 Coexpressed gene neighborhoods promote expression of newly created genes through chromatin architecture sharing in *Drosophila* *Kirill Borziak*[1], Elaine Rettie[2], Steve Dorus[1]. 1) Dept of Biology, Syracuse University, Syracuse, NY; 2) Department of Biology and Biochemistry, University of Bath, Bath, UK.

Eukaryotic genomes are highly organized both in terms of gene order and chromatin structure. Research has established a clear relationship between chromatin architecture and gene expression on a local level, however, the evolutionary ramifications of this relationship on gene order and gene creation have not been fully explored. To gain additional insight into these interactions, we performed a comprehensive, genome-wide analysis of coexpressed gene clusters (CGCs) revealing a strong interplay between gene order, chromatin architecture, and expression profiles of new genes. Using two independent clustering algorithms on the *Drosophila melanogaster* genome, we identified 598 CGCs that cover over 25% of the genome, with significant enrichment (1.43-fold) of testis-enriched CGCs. Supporting the broad scale interaction between chromatin and gene expression, tissue-enriched CGCs, especially testis CGCs, were predominantly and significantly associated with repressive chromatin in an embryonic stem cell line, while ubiquitous and broadly expressed CGCs were predominantly associated with an open chromatin conformation. Supporting their role in new gene creation, we find testis retrogenes to be significantly associated with testis CGCs. Further, retrogenes have a strong adoption of the chromatin architecture and expression profiles of their containing CGCs, especially those located within testis CGCs. These results suggest that chromatin architecture influences the evolution of expression of new genes, and by extension further supports it's role in the establishment of CGCs across evolutionary time.

33 Natural variation in color perception in flies *Caitlin Anderson*[1], India Reiss[1], Cyrus Zhou[1], Annie Cho[1], Haziq Siddiqi[1], Ben Mormon[2], Cameron Avelis[1], Elijah Roberts[1], James Taylor[1], Dan Vasiliauskas[3], Robert Johnston[1]. 1) Biology, Johns Hopkins University, Baltimore, MD; 2) Biology, New York University, New York, New York; 3) Université Paris Diderot, Paris, France.

The genetic and molecular mechanisms underlying natural variation in color perception are poorly understood. The fly eye, similar to the human eye, is a random mosaic of color-detecting photoreceptors. In flies, the random pattern is determined by the stochastic on/off expression of the transcription factor Spineless (Ss). Individual R7 photoreceptors stochastically choose between Ss on and Ss off fates, resulting in unique patterns but consistent proportions of cell types in genetically identical flies. Here, our studies of natural variation identified a single nucleotide insertion in a non-coding DNA element in the *ss* gene locus (*"spineless insertion" or* "*sin*") that lowers the ratio of Ss on to Ss off R7s. This change in photoreceptor ratio alters color perception, as flies with *sin* have a stronger innate preference for blue light than flies without *sin* that prefer green light. *sin* increases the binding affinity for the Klumpfuss (Klu) zinc finger transcriptional repressor that acts in R7s to lower the ratio of Ss on to Ss off cells. Increasing Klu levels causes a decrease in the proportion of Ss-expressing R7s whereas loss of *klu* causes more R7s to express Ss. Our data suggest that transcription factor levels and binding site affinity together control stochastic on/off gene expression, setting the ratio of alternative cell fates and ultimately determining color perception.

34 Translational regulation by ATF4-induced 4E-BP is essential for the innate immune response *Deepika Vasudevan*, Jessica Sam, Hyung Don Ryoo. Department of Cell Biology, New York University School of Medicine, New York, NY.

In response to intrinsic or external stresses, cells often attenuate translation by activating specific inhibitors of translational initiation. Recovery from such stress usually requires the expression of new transcripts, but it remains unclear whether and how those stress-responsive transcripts undergo active translation in the presence of translational inhibitors. Here, we report that in response to bacterial infection, the stress-response kinase GCN2 lies upstream of the translational inhibitor 4E-BP, which selectively permits the translation of immune-responsive transcripts. Specifically, we found that 4E-BP (*thor* in *Drosophila*) is transcriptionally induced during infection with a bacterial pathogen Ecc15, and this induction was suppressed in mutants for ATF4, a transcription factor downstream of GCN2. Additionally, GCN2, ATF4, and 4E-BP mutants infected with Ecc15 showed immune-compromised phenotypes. The intron of 4E-BP ($4E\text{-}BP^{i}$) has several ATF4 binding sites based on which we generated a dsRed reporter driven by $4E\text{-}BP^{i}$. Upon oral infection with Ecc15, this reporter expression was elevated in the gut and fat body, corroborating our data that ATF4 induces 4E-BP in response to infection.

A key output of the *Drosophila* innate immune response is the synthesis of anti-microbial peptides (AMPs), and therefore we examined the relationship between 4E-BP and AMPs. While the transcript levels of AMPs were unchanged in 4E-BP mutants, the amount of the AMP Attacin was reduced in the hemolymph of these organisms. Since 4E-BP inhibits the initiation factor eIF-4E, which mediates the translational initiation of most cellular transcripts, we asked whether 5'UTRs of AMP transcripts could evade regulation by 4E-BP. We found that the 5'UTR of the AMPs scored positively in a bicistronic assay, suggesting that this transcript can bypass the requirement of eIF-4E. We thus propose that AMPs not only have mechanisms to evade translational inhibition during infection-induced stress, but may also require this inhibition of translation initiation by GCN2/ATF4/4E-BP for their efficient translation.

35 Positive and negative functions of Polycomb binding sites in the *vestigial* gene region *Kami Ahmad*, Amy Spens, Charlotte Schubert, Tayler Hentges. Division of Basic Sciences, FHCRC, Seattle, WA.

Polycomb-mediated silencing is critical for the control of developmental gene expression programs. Short *Polycomb Response Elements* (*PRE*s) are essential to establish and maintain repressed domains, but how Polycomb silencing is integrated with developmental signalling and transcriptional enhancers is unknown. We used high-resolution chromatin profiling to dissect the architecture of two *PRE*s near the developmental selector gene *vestigial*, a gene with five characterized enhancers in a ~40 kb Polycomb-regulated domain. We found that a *PRE* near the promoter (*pPRE*) contains strong factor-binding sites that anchor Polycomb near a paused RNA Polymerase II (RNAPII) complex. This architecture is typical of many Polycomb-bound promoters in Drosophila. Transgenes with the *pPRE* show pairing-sensitive silencing, but surprisingly directed mutations demonstrate that this element is required for *vestigial* expression. We suggest the *pPRE* is required to pause RNAPII just downstream of the promoter. While in repressed cells pausing suffices to silence *vestigial*, in wing progenitor cells developmental signals release paused RNAPII to induce *vestigial* expression. In contrast, the second distal *PRE* (*dPRE*) has a very different architecture, where three juxtaposed but weak factor-binding sites anchor Polycomb to DNA. Switching between silenced and de-repressed cells corresponds to binding of the factor-Polycomb complex at distant *PREs*. We suggest that poor sequence motifs for transcription factors are a critical 'design principle' for regulatory elements to switch during developmental transitions. Finally, directed mutagenesis identified additional elements around the *dPRE* that appear to shut off the *dPRE* in wing progenitor cells. We are now defining how mutations at the *pPRE* and *dPRE* affect the activity of enhancers and chromatin states in the *vestigial* domain.

36 Who is the Shadow? Developmental Shadow Enhancers Come in Two Flavors, Only One of Which Is Targeted by Su(H) and Other Polyglutamine-Rich Factors Timothy Fuqua[1], Clinton Rice[2], Elizabeth Stroebele[3], *Albert Erives*[1]. 1) Department of Biology, University of Iowa, Iowa City, IA 52242-1324, USA; 2) Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Ave., Lawrence, KS 66045, USA; 3) Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX, 75390-8511, USA.

"Shadow" enhancers are additional *cis*-regulatory DNAs from a gene locus that drive "redundant" expression patterns similar to other enhancers at the locus. Shadow enhancers are thought to provide developmental robustness. Such redundant enhancer activities are necessary when cells are subjected to atypical conditions, such as heat stress or haplo-insufficiencies. Nonetheless, proposed hypotheses of evolutionary selection for robustness under stress do not indicate how shadow enhancers are constructed. Possibly, redundant enhancers evolve by *parallelism* such that they contain the same *cis*-elements for a common set of tissue selectors and signaling effectors. Alternatively, redundant enhancers evolve by *convergence* such that they contain distinct *cis*-elements for non-overlapping sets of factors, which can drive approximately similar expression patterns. Here we show that redundant enhancers are constructed from distinct enhancer archetypes in two different developmental contexts in *Drosophila*: (*i*) Stage 5 neuroectoderm, and (*ii*) Stage 10 tracheal placodes. Our work on tracheal placode

enhancers (TPEs) is based on our discovery of the *rhomboid* (*rho*) TPE. For each of these two embryonic contexts, one of the two enhancer archetypes has a sparse *cis*-element composition that does not include binding sites for the Notch signaling effector Su(H). In contrast, a second archetype corresponds to Su(H)-dependent enhancers that are targeted by a large set of distinct polyglutamine-rich factors, including the Zelda pioneer factor. These results demonstrate that superficially redundant enhancers are not mechanistically equivalent and interchangeable but rather are interlocking modules of a locus-wide apparatus, each module of which responds independently to separate signaling pathways.

37 Dual Readout of Regulatory Information Is a Common Feature of Transcriptional Silencers *Stephen Gisselbrecht*[1], Alexandre Palagi[1,2], Jesse Kurland[1], Martha Bulyk[1,3]. 1) Division of Genetics, Brigham & Women's Hospital, Boston, MA; 2) Doctoral School of Health and Life Sciences, University of Nice, France; 3) Departments of Medicine & Pathology, Harvard Medical School, Boston, MA.

Transcriptional regulation is a complex, fundamental mechanism responsible for the proper spatio-temporal control of gene expression relying on two kinds of cis-regulatory modules, enhancers and silencers. While enhancers have been relatively well studied, silencers remain challenging to study and thus somewhat poorly understood. We have developed a two-step strategy to screen hundreds of sequences for tissue-specific silencer activity in *Drosophila melanogaster* embryos. Our analysis showed that the only sequences that functioned as mesodermal silencers were enhancers in other cellular contexts. These bifunctional elements were enriched in HOT region overlap, specific TF motif combinations, and histone H3 K27 trimethylation. The rate at which known enhancers demonstrate silencer activity in alternate contexts suggests the possibility that thousands of these bifunctional elements may remain to be discovered in *Drosophila*.

38 A double assurance mechanism controls enhancer-promoter specificity at the *hunchback* locus *Jia Ling*, Stephen Small. Department of Biology, New York University, New York, NY.

Spatiotemporal gene expression is determined by enhancers, which interact with basal promoters to activate transcription. In cases where multiple enhancers and promoters co-exist at the same gene locus, individual enhancers target their specific promoters to activate. However, the underlying mechanism that regulates this enhancer-promoter interaction is unknown. In the current study, we focused on *hunchback* (*hb*), which contains two promoters: P1P, a maternal and late zygotic promoter, and P2P, an early zygotic promoter, and two *Bicoid* (*bcd*)-dependent enhancers: the proximal enhancer and the distal enhancer. The distal enhancer is located 4kb upstream of P2P but is close to P1P. By using a reporter system that mimics the endogenous *hb* locus and single-molecule FISH (smFISH) technique capable of quantifying number of transcripts from the two promoters, we found that the distal enhancer bypasses P1P and activates the more remote promoter P2P, and that the proximal enhancer also interacts with P2P, but not P1P. To investigate how these specific interactions between promoters and enhancers are controlled, we tested two hypothetical mechanisms: (i) motifs within the targeted promoter guide each enhancer to its specific promoter, and (ii) chromosome architecture, which facilitates contacts between distal and proximal enhancers with P2P while blocking P1P. By manipulating DNA sequences in our reporter system, we show that inserting a TATA box with a Zld site to P1P can significantly elevate the level of its expression, but chromosome architecture plays a role in preventing P1P from being activated as well. These results suggest that promoter motifs do affect a promoter's interaction specificity with an enhancer, but whether an interaction happens *in vivo* also depends on the chromosome environment. Overall, our experiments provided evidence for a double-assurance mechanism that provides robust expression of P2P to achieve normal thoracic development.

39 Gap junctions are required for glia-glia communication, calcium signaling and survival in *Drosophila* peripheral nervous system (PNS) *M. Das*, T. Matzat, X. Xi, V. Auld. Zoology, University of British Columbia, Vancouver, British Columbia, Canada.

The *Drosophila* PNS harbors the axons of sensory and motor neurons connecting the central nervous system to the periphery. To ensure the proper functioning of neurons, axons in the PNS are surrounded by three distinct glial layers. The innermost glial layer is the wrapping glia (WG) that directly contacts axons. The middle layer formed by the subperineurial glia (SPG) acts as a paracellular diffusion barrier through the formation of septate junctions (SJs) analogous to the blood-brain barrier in vertebrates. The outermost layer is the perineurial glia (PG) covering the whole nerve and is encircled by a layer of extracellular matrix. Extensive communication between these different glia layers is required for proper development of peripheral nerves as they undergo extensive growth and differentiation, which has to be tightly coordinated. However, the molecular pathways underlying these regulatory mechanisms are not known.

One mechanism by which cells can communicate is via gap junctions (GJs). We have shown that a GJ protein, Innexin 2 (Inx2) is present in all three glial layers in the *Drosophila* PNS. Similar to *inx2* null mutants, which are embryonic lethal the knockdown of Inx2 in all glial cells leads to lethality during larval stages. To determine which glial layers require Inx2, we knocked down Inx2 in each individual glial layer using specific GAL4 lines. The loss of Inx2 in

PG showed no effect, whereas the knockdown of Inx2 in the SPG resulted in the disruption and death of the WG, indicating a non-autonomous effect. As GJs are known to transmit calcium signals across cells, we wanted to test if Ca^{2+} mediates the disruption of the WG across the SPG non cell autonomously. Therefore, we imaged calcium signals in individual PNS glia using the GCaMP sensors. Ca^{2+} transients were observed in both the SPG and the WG of wild type larvae, whereas these transients were absent after Inx2 RNAi mediated knockdown in both the SPG and WG. The knockdown of the IP3 receptor also blocked calcium transients indicating that IP3 mediated release of Ca^{2+} is one mechanism of calcium signaling in the peripheral glia. However, loss of IP3 signaling in the SPG did not result in WG disruption, suggesting that although calcium transients are present in the SPG, this signal is not the survival signal mediated by Inx2 from the SPG to WG. Interestingly, individual knockdown of Inx2 and the IP3 receptor in the WG, both resulted in disruption and death of WG, implying that Inx2 mediated calcium is required within the WG itself. In summary we propose that Inx2 mediates an unknown signal between the SPG and the WG whereas, calcium is mediated by Inx2 between WG-WG and both signals are required for the survival of the WG.

40 A pulsatile EGFR signalling in the neighbouring somatic cells sets the pace of germ cell divisions in *Drosophila* testis *Purna Gadre*, Bhavna Varshney, Shambhabi Chatterjee, Chetanchandra Joshi, Samir Gupta, Krishanu Ray. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India.

Cellular microenvironment plays a pivotal role in both promoting and terminating the transit amplifying divisions of stem cell progeny. For example, EGFR signalling between the gonialblast cell and the surrounding cyst cells of somatic origin play a crucial role in managing the spermatogonial divisions in *Drosophila* testis. Loss of EGFR signalling in the somatic cyst cells results in germline over-proliferation. Though the gross effect of perturbing the EGFR signalling is known, the mechanism is still unclear. We enumerated the distribution of transit amplifying cysts of different stages in adult testis in wild-type controls and after temporally controlled somatic activation of EGFR. The results indicate that the periods of germline divisions progressively shorten in successive stages in wild-type backgrounds. Also, the data analysis suggested that an enhancement of somatic EGFR signalling could proportionally slow down the cell division rates causing a progressive decline in the number of germline cysts at all stages. Investigation of the ERK phosphorylation patterns in the somatic cells further suggested that the EGFR activation occurs for a brief period at every stage of germline divisions. We found that the secretion of an EGF ligand (*spitz*) from the germline cells and a battery of feedback regulators induced by the EGFR activation in somatic cells define the phosho-ERK pulses. Alteration of the pulse durations using selective knockdown of certain feedback inhibitors in the somatic cells altered the relative distribution of the germline cysts. Altogether, the results suggest that a pulsatile EGFR activation in the somatic cyst cells could define the periods of neighbouring germ cell divisions in *Drosophila* testis.

41 Phosphorylation Potential of *Drosophila* E-Cadherin Intracellular Domain is Essential for Development and Regulating Adherens Junction Biosynthetic Dynamics *Yang Hong*, Yi-Jiun Cheng, Juan Huang, Lynn Huang. Dept Cell Biol & Physiology, Univ Pittsburgh Med Sch, Pittsburgh, PA.

E-Cadherin intracellular domain contains a highly conserved serine cluster whose phosphorylations are essential for binding to β-Catenin in vitro. In cultured cells phosphorylations of specific serine residues within the cluster are also required for regulating adherens junction (AJ) stability and dynamics. However, much less is known how such phosphorylations of E-Cadherin regulate the AJ formation and dynamics in vivo. In this report we generated an extensive array of *Drosophila* E-Cadherin (DE-Cad) endogenous knock-in alleles that carry mutations targeting this highly conserved serine cluster. Our mutant analyses suggest that the overall phosphorylation levels, other than the site-specific phosphorylations, of serine cluster enhance the recruitment of β-Catenin by DE-Cadherin in vivo. Moreover, phosphorylation potential of the serine cluster only moderately increases β-Catenin in AJ complex and is in fact dispensable for AJs formation in vivo. However, phosphorylation-dependent recruitment of β-Catenin is essential for development, likely by enhancing the interactions between DE-Cad and α-Catenin. Specific phospho-mutations can also dramatically affect the biosynthetic turn-over of mutant DE-Cad during apical-basal polarization and such mutants specifically rescued the polarity defects in embryos lacking polarity protein Stardust(Sdt) and Crumbs(Crb).

42 Actomyosin contractility modulates Wingless signaling through adherens junction stability *Eric Hall*, Elizabeth Hoesing, Endre Sinkovics, Esther Verheyen. Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

Mechanical force and cytoskeletal tension are increasingly being identified as key factors in promoting and regulating signal transduction pathways during development, and tissue homeostasis. Mechanical force has been identified to perturb Wnt signal transduction in developing tissue, yet an identified molecular mechanism remains elusive. In an *in vivo* RNAi screen designed to identify novel phosphatase and kinase regulators of the Wnt/Wingless (Wg) pathway in developing *Drosophila,* we established that the reduction of myosin phosphatase components, protein phosphatase type 1β (PP1β)[encoded by the *flapwing (flw)* gene] and *myosin phosphatase targeting protein* (*Mypt*)*-75D*, disrupted Wg signaling. Mypt-75D and PP1β are known to function together to dephosphorylate

and inactivate the actomyosin contractile motor protein, non-muscle myosin II (NM II). Knockdown of *flw* and *Mypt-75D* in epithelial tissue reduced Wg target gene expression, but did not affect Wg ligand expression or stability. The destruction complex, which degrades the Wg pathway's key effector protein Armadillo (*Drosophila* β-Catenin), maintained its localization to the cell membrane under active Wg conditions. In these cells Armadillo failed to accumulate in the cytoplasm and nucleus, and was preferentially enriched at the adherens junctions (AJ). Tissue expressing *flw-RNAi* and *Mypt-75D-RNAi* exhibited elevated NM II activation and corresponding cellular contraction. Gain or loss of function mutations of NM II, resulted in similar Wg pathway disruption. These defects were also seen in mammalian cell culture experiments. Subsequent epistasis experiments confirmed Wg defects associated with loss of myosin phosphatase were mediated through increased activation of NM II. Genetic interaction experiments confirmed actomyosin contractility directly regulated Wg activity, and was not indirectly mediated through other signal transduction pathways. Expression of activated NM II or constitutively active formin proteins resulted in increased filamentous (F)-actin and accumulation of AJ proteins to the AJs, including Armadillo/β-Catenin the key linker protein of the AJs. This effect could be rescued by reducing levels of F-actin and correspondingly would rescue the loss of Wg target gene expression. From these results we have identified a mechanism in which actomyosin contractility modulates Wnt pathway activation, by sequestration of Armadillo/β-Catenin proteins to the AJ. This compensatory mechanism may be a physiological response to preferentially maintain tissue integrity over growth and patterning during development and morphogenesis.

43 Actomyosin contractility is required for long-distance Notch signaling *Ginger Hunter*[1,2], Li He[3], Lenny Campanello[4], Guillaume Charras[5], Ed Giniger[1], Buzz Baum[2]. 1) NINDS/NIH, Bethesda, MD; 2) MRC-LMCB, University College London, London, UK; 3) HHMI, Dept of Genetics, Harvard Medical School, Boston, MA; 4) Dept of Physics, University of Maryland-College Park, College Park, MD; 5) Dept of Cell and Developmental Biology, University College London, London, UK.

Here we investigate the mechanism by which Notch signaling occurs at long distances (≥ 1 cell diameter), via actin-rich, filopodia-like, basal protrusions, to drive cell fate decision making during wild type bristle pattern formation in the Drosophila thorax.

We find that Notch receptor and Delta ligand localize to basal protrusions, which are dynamic structures on the basal surface of pupal thorax epithelium (notum) and are required for wild type bristle spacing. The activity and morphology of basal protrusions and basal cytoskeleton is regulated by non-muscle myosin 2 (NMM2); furthermore, loss of NMM2 activity in bristle precursor cells leads to defects in precursor spacing, indicating that long-distance signaling is perturbed.

Our data show that NMM2 activity, in parallel with endocytosis, can regulate Notch activation during basal protrusion-mediated lateral inhibition. In vitro and in cell culture, mechanical force is required to activate Notch signaling, via exposure of an extracellular cleavage site. For adjacent cells, ligand endocytosis provides this force. When NMM2 activity is lost in precursor cells alone, there is a decrease in Notch response (via transcriptional reporter expression) in adjacent and distant epithelial cells. When NMM2 activity is decreased in all cells, Notch response is decreased in cells signaling at a distance only. We simultaneously knocked down NMM2 activity and endocytosis in S2 cell culture, using small molecule inhibitors and RNAi; this resulted in decreased Notch response. Tissues co-expressing RNAi against NMM2 heavy chain and ligand endocytosis in precursor cells have disrupted bristle patterns, indicating that NMM2 activity and endocytosis have overlapping functions for Notch activation in vivo.

44 Mechanical Stress Regulates Insulin Sensitivity Through Integrin-dependent Control of Insulin Receptor Localization. *J. Kim*[1,2], D. Bilder[1], TP. Neufeld[2]. 1) Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA; 2) University of Minnesota, Minneapolis, MN.

'Insulin sensitivity' describes the cellular activity of the Insulin pathway in the presence of ligand, and misregulation of Insulin sensitivity leads to metabolic diseases including type-2 diabetes. Mechanical stress resulting from e.g. exercise is a known regulator of Insulin sensitivity, but the underlying molecular mechanisms are unclear. Here, we use the Drosophila larval fat body to address this question. We find that Insulin signaling activity is abolished in the absence of mechanical stress, even when excess Insulin is present. Mechanical stress is required for activation of Insulin signaling in a new *ex vivo* assay as well as *in vivo*. Interestingly, Insulin Receptor (InR) and other downstream InR substrates are cytoplasmically localized in the absence of mechanical stress, but are recruited to the plasma membrane upon stress initiation. Stress sensing is mediated by Integrins, whose activation is sufficient as well as necessary for recruitment and signaling thought InR; Integrin activation is also necessary and sufficient for mechanical stress-dependent activation of Tor. Together, our data suggest that Integrin-mediated mechanical stress controls Insulin sensitivity by altering localization of InR.

45 Occluding junctions regulate Hippo signalling to control blood cell differentiation in *Drosophila* *Rohan Khadilkar*, Guy Tanentzapf. Tanentzapf lab, Department of Cellular and Physiological Sciences, Life Sciences Institute, Vancouver, British Columbia, Canada.

Hematopoiesis in *Drosophila* involves the precise co-ordination of multiple cell signaling pathways to control the proliferation and differentiation of cells derived from progenitor cells called prohemocytes. The evolutionary conserved Salvador-Warts-Hippo signaling pathway, which is known to regulate tissue growth and organ size, has been shown to be a key regulator of fly hematopoiesis. Specifically, disruption of Hippo pathway genes gives rise to defects in hemocyte differentiation and proliferation in the lymph gland, the *Drosophila* hematopoietic organ. A central feature of the Hippo pathway is its ability to integrate and respond to inputs from diverse cell biological parameters such as biomechanical cues, cell polarity, and cell contacts. Here we show that Cell-Cell interaction via septate junctions acts as an additional, novel, regulatory input that regulates the Hippo pathway in the context of fly hematopoiesis. We noted that loss of septate junction components in the medullary zone of the lymph gland, which contains the prohemocytes, resulted in phenotypes that were strikingly similar to those seen in Hippo pathway mutants such as a drastic increase in organ size and hemocyte proliferation. Loss of the septate junction component Coracle resulted in increased crystal cell specification, mixed lineage fate, and blood cell proliferation, which are all hallmarks of deregulation of the hippo pathway. Loss of Coracle also triggers markers for Hippo pathway deactivation. Systematic analysis of epistatic interactions between septate junction and Hippo pathway mutants revealed that septate junction components genetically interact with, and function upstream of, the Dachsous-Fat signalling module. Importantly, loss of Yorkie, which is functionally activated by mutations in the Hippo pathway, suppresses the lymph gland phenotypes caused by loss of Coracle. Intriguingly, although genetically Coracle acts upstream of *fat* it fails to localize to the membrane in *fat* mutants suggesting a possible association between these proteins. Consistent with this, previous proteomic approaches identified direct binding between Fat and Cora. Our findings identify a novel mechanism of junctional control of Hippo signalling in cellular differentiation during fly hematopoiesis.

46 Pharmaceutical inhibition of MEK/ERK cascade alleviates tumor-induced wasting effects *Wei Song*[1], Shangyu Hong[2], Alex Banks[2], Norbert Perrimon[1]. 1) Genetics, Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA.

Cross-talk between tumors and host tissues plays essential roles in cancer cachexia or tumor-associated systemic wasting, including muscle wasting and lipid loss. However, the pathogenic molecular mechanism(s) of wasting is still poorly understood. Using a fly cancer cachexia model, we demonstrate that MEK/ERK signaling is aberrantly activated in the fat and muscle tissues of flies bearing gut Yorkie-tumors in a cell-non-autonomous manner. Specific genetic activation of MEK/ERK cascade in the wild-type muscle and fat body results in muscle wasting and lipid loss, respectively. Strikingly, feeding tumor-bearing flies with low dose of MEK inhibitors moderately suppresses MEK/ERK signaling in the fat body and muscle and significantly alleviates cachectic effects, including muscle wasting, lipid loss, hyperglycemia and mortality, without affecting tumor progression. Moreover, when we generated tumors resistant to MEK inhibitors via overexpression of an active ERK and fed flies high dose of MEK inhibitors, a potent suppression of MEK/ERK signaling in fat and muscle tissues was observed and the cachexia effects were completely suppressed in the context of tumor progression. Conserved beneficial effects were also observed in mammalian wasting models, as MEK inhibitors improves lipid loss in adipocytes and muscle atrophy induced by conditioned medium from cachectic cancer cells. Thus, our results demonstrate the essential roles of MEK/ERK in tumor-induced wasting and indicate that MEK/ERK inhibitors could be used for treatment of cancer cachexia.

47 Sestrin is required for exercise adaptations of flies and mice Myungjin Kim[1], Alyson Sujkowski[2], Jun Hee Lee[1], *Robert Wessells*[2]. 1) 1Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109, USA; 2) Dept of Physiology, Wayne State University School of Medicine, Detroit, MI.

Exercise induces stress responses that evoke hormetic effects on the metabolic fitness of many organisms, including humans. As a stress-inducible modulator of both oxidative stress and target of rapamycin (TOR) signaling, Sestrin may be involved in mediating exercise-induced hormesis. Here we present genetic evidence supporting a biological role for Sestrin in mediating exercise adaptation. Sestrin-deficient flies and Sestrin1-deficient mice exhibited defective responses to exercise, failing to expand mobile capacity or improve insulin signaling sensitivity. In flies, muscle-specific overexpression of Sestrin strongly extended endurance and mobility across ages, even without exercise intervention. The Sestrin-induced mobility extension was mainly through activation of TOR complex 2 (TORC2)/AKT signaling, while oxidative damage reduction or TORC1 inhibition played a relatively minor role. In addition to potentiating insulin-AKT signaling upon exercise, mouse Sestrin1 is also important for exercise-induced improvement of respiratory efficiency. These results highlight the potential of Sestrin as a target for exercise-mimicking therapeutics.

48 Key molecular regulators maintain metabolic and development balance during temperature fluctuations *Steven G Kuntz*[1,2], Anthony T Iavarone[3], Peter A Combs[5], Michael B Eisen[2,3,4]. 1) Ionis Pharmaceuticals, Carlsbad, CA; 2) Molecular and Cell Biology, UC Berkeley, CA; 3) QB3, UC Berkeley, CA; 4) HHMI, Berkeley, CA; 5) Stanford University, CA.

Frequently discounted as trivial, the developmental response to temperature is a prime example of adaptive evolution so streamlined that it has been largely ignored. Despite speculation that uniform morphological scaling of embryogenesis is a natural consequence of physical laws, we have demonstrated that this process has evolved and is malleable, much like the developmental response to hypoxia. However, while both temperature and oxygen-levels shift development, the mechanism for temperature-induced shifts in growth and development have been entirely undescribed. Even though the oxygen-dependent mechanism is known, the temperature response is both fundamentally different and responds independently to permit a great deal more flexibility in developmental control.

Through precision in temperature control and developmental staging, we profiled the expression and metabolic profiles of developing embryos across a wide range of temperatures (17.5-30°C). We observed major changes in amino acid, nucleotide, and glycolysis metabolism. This was in contrast to precise maintenance of developmental scaling and gene expression. In fact, only a handful of genes changed, and a majority of those twenty-five genes are key regulators or players in major cellular processes, including transcription (*Myc*), translation (*Thor*), lipid detection (*LRP1* and CG8507), metabolism (*Sirup*, *scully*, *pAbp*, *Argk*, *awd*, CG5028, CG6439, CG9231, and CG16758), cell death (*Dronc* and *Hsp23*), and growth regualtion (*charybde* and *scylla*). Additionally, important signaling genes also changed with temperature (*Art1*, *Gbeta13F*, *moleskin*, *Nipped-A*, and *Ubc10*). This is reflected in the maintenance of major developmental processes despite changes in polysome lengths with temperature. These data strongly suggest that the cellular responses act to mediate discrepancies that inevitably arise between different components as temperatures change.

49 Reversal of Hyperactive Wg Signaling-Dependent Fat Body Defects by Peptide Boronic Acids Tianyi Zhang[1], Fu-Ning Hsu[2], Xiao-Jun Xie[2], Xiao Li[2], Xinsheng Gao[2], Xun Pei[1], Yang Liao[1], Wei Du[1], *Jun-Yuan Ji*[2]. 1) Ben May Department for Cancer Research, The University of Chicago, 929 E. 57th Street, Chicago; 2) Texas A&M Health Science Center, College Station, TX.

Although critical roles of the Wnt signaling cascade in regulating normal development and tumorigenesis have been well appreciated, its function in regulating fat metabolism is less understood. Given that hyperactivated Wnt signaling can lead to drastic defects in adipocyte function, medicinal intervention might be beneficial to patients with deregulated Wnt activities. To address these two issues, we have established a *Drosophila* model with hyperactivated-Wnt (Wingless or Wg in *Drosophila*) signaling caused by partial loss of Axin (Axn). We find that *Axn* mutant larvae are transparent, accompanied by severe reduction of fat accumulation. At the cellular level, the abdominal adipocytes in *Axn* mutant larvae remain small during the third instar larval stage and fail to accumulate triglycerides. These defects are caused by ectopic accumulation of Armadillo (β-catenin in mammals), upregulation of Wg signaling target genes, and defects in both adipose tissue and gut. These hyperactivated Wnt signaling phenotypes micmic multiple diseases in humans. Through screening a compound library and subsequent analyses, we identified proteasome inhibitor Bortezomib (BTZ) as well as additional peptide boronic proteasome inhibitors that can potently rescue the adipocyte defects of *Axn* mutants. Mechanistically, BTZ suppresses Wg activities by stabilizing α-catenin: ectopic expression of α-catenin is sufficient to rescue the adipocyte defects in *Axn* mutants; conversely, depletion of α-catenin in adipose tissue abolished the rescuing effects of BTZ on *Axn* mutants. These results suggest that peptide boronic acids such as BTZ can preferentially stabilize α-catenin, which inhibits the extra β-catenin caused by *Axn* mutation, thereby attenuating β-catenin-stimulated transcription and rescuing defective fat metabolism in *Drosophila*. These findings indicate that, instead of developing inhibitors that directly target the canonical Wnt signaling pathway, pharmacological modulation of β-catenin activity through α-catenin is an attractive approach to attenuate Wnt signaling *in vivo*.

50 Bacterial Vitamin B6 Metabolism Promotes *Drosophila melanogaster* Lifespan on Calorie-Rich Diet *Melinda L Koyle*, Madeline Veloz, Corinne Penrod, Bethany Banks, John Chaston. Plant and Wildlife Sciences, Brigham Young University, Provo, UT.

Statement of Purpose: Animal-associated microbes ('microbiota') exert substantial influence on animal development, including aging. However, the interactions between microbes and established mechanisms of aging, including calorie and methionine restriction, are poorly understood. To begin to dissect these different effects we sought to identify bacterial genes that influence lifespan of *Drosophila melanogaster*, a key model in aging research and a developing model for host-microbiota interactions.

Methods: We developed a three-step approach to identify microbial genes that influence fruit fly lifespan. First, we measured the individual influence of 41 genome-sequenced bacterial strains on *D. melanogaster* lifespan when the flies were reared on a nutrient rich diet. Flies were transferred to new vials every 2-3 days to prevent poor diet conditions. Second, we predicted bacterial genes that influence *D. melanogaster* lifespan by metagenome-wide association (MGWA) that compared the presence of bacterial genes with *Drosophila* lifespan effects. Finally, to confirm the MGWA predictions we measured lifespan of flies reared in monoassociation with bacteria bearing mutations in a few top predicted genes.

Results: MGWA identified two bacterial pathways that influence *D. melanogaster* lifespan on a calorie-rich diet.

Since fruit fly longevity was significantly influenced by the presence of different bacterial species and their persistence with the flies, we accounted for bacterial identity and persistence in our statistical model. 129 genes were predicted to influence fruit fly lifespan, including vitamin B metabolism genes. When we measured lifespan of flies reared from birth with bacterial transposon insertion mutants for the predicted genes, we confirmed roles for bacterial vitamin B5 and B6 metabolism in fly longevity.

Conclusion: Our working model is that disrupting microbial vitamin B6 biosynthesis influences the abundance of specific *D. melanogaster* metabolites, including methionine, with consequent effects on animal methionine restriction and lifespan. To test this hypothesis we are currently measuring vitamin and amino acid levels in fruit flies associated with the different bacterial mutants. We anticipate that understanding how microbes influence *D. melanogaster* lifespan may guide development of rationally-designed therapeutics for lifespan extension in mammals, including humans, that are independent of calorie restriction.

51 An Autonomous Metabolic Role for Split Ends in *Drosophila melanogaster* *Kelsey Hazegh*, Travis Nemkov, Angelo D'Alessandro, Jen Monks, Elise Bales, Jim McManaman, Kirk Hansen, Tania Reis. University of Colorado Anschutz Medical Campus, Aurora, CO.

Genetic background plays a major role in obesity. However, only a few of the responsible genes have been identified. We previously developed a novel, buoyancy-based assay for body fat in *Drosophila* larvae and used it in an unbiased forward genetic screen to identify *split ends* (*spen)* as a new fat regulator. Spen is an RNA-binding protein previously implicated in transcriptional control of conserved signaling pathways. We find that Spen function is necessary and sufficient to promote fat depletion in the fat body (FB) in a cell autonomous manner. Interestingly, despite being fat, larvae in which Spen is depleted from the FB are sensitive to starvation, suggesting that these animals are incapable of using their excess fat stores. Consistent with this phenotype, metabolomics and RNAseq demonstrate metabolic alterations in Spen-depleted FBs indicative of a defect in mobilization of energy stores, and utilization of other metabolites (proteins and carbohydrates) as primary sources of energy. We further find that another Spen family member, Spenito (Nito), plays an opposing role in fat storage. FB overexpression of an N-terminal Spen fragment containing the RNA Recognition Motifs (RRMs) causes a dominant-negative high-fat phenotype, whereas there was no effect of overexpression of a C-terminal fragment lacking the RRMs but containing a nuclear localization signal and the conserved Spen paralog and ortholog (SPOC) domain. Thus, the RRMs are required for the ability of overexpressed full-length Spen to deplete fat stores, and when overexpressed alone may sequester important Spen-associated RNAs into non-functional complexes. We propose that Nito, which contains RRMs and a SPOC domain but is much smaller than Spen, may act as a negative regulator of Spen function. We further find that levels of the mammalian Spen ortholog correlate with body weight in a diet-induced obese mice, supportive of a model where Spen normally regulates lipolysis. Consistently, mammalian adipocytes transfected with mSpen accumulate fewer and smaller lipid droplets. No other study has implicated Spen in the regulation of metabolism or body fat control. Our work provides a new direction for understanding metabolic disease as well as a molecular handle to generate novel mechanistic insights into conserved causes of obesity.

52 The Gut as an Adaptable Interface: from Genetic Architecture to Physiological Consequences of Adaptive Growth of the *Drosophila* Gut. *Alessandro Bonfini*[1], Adam Dobson[2], David Duneau[3], Xi Liu[1], Philip Houtz[1], Jonathan Revah[1], Matthew Piper[4], Nicolas Buchon[1]. 1) Entomology, Cornell University, Ithaca, NY, USA; 2) Institute of Healthy Ageing, University College London, London, UK; 3) Paul Sabatier University - Toulouse III, Toulouse, France; 4) Monash University, Victoria, Australia.

Nutrition is a principal determinant of animal health and fitness, affecting aging, metabolic diseases and fecundity. Thus, fine-tuning the balance of dietary nutrients has profound effects on organismal health and fitness. The gut acts as the primary interface between food and an organism, and is involved in nutrient processing and uptake. Surprisingly, the gut interface is itself variable and the gut tissue alters its structure in response to changes in diet, a process termed “adaptive growth”. We found that guts of flies raised on isocaloric diets with distinct ratios of yeast to sucrose vary in size and structure. Guts growing on a High Yeast (HY) diet were 40% longer than guts growing on High Sugar (HS) diet. We utilized the nutritional geometrical framework to explore 20 diets with both varying caloric content and Yeast:Sucrose ratios, and identified the complex relationship between nutrient composition and gut structure. We further analyzed the cellular changes responsible for gut structure alteration and found that gut growth occurs as a combination of increase in enterocyte size and numbers, as well as altered stem cell activity. The analysis of gut growth in response to food in 197 isogenic lines of the *Drosophila* genetic resource panel (DGRP) showed strong inter-individual differences. Transcriptomic data of the gut on different food and during gut growth, as well as Genome Wide Association study allowed the identification of key genes controlling adaptive growth in *Drosophila*. Here, we will present new data on the mechanisms controlling adaptive gut growth, and their impact on host fitness. Our results demonstrate that adaptive gut growth, and its genetic determinants, have an important role in determining the nutritional physiology of the host, an important and overlooked aspect of nutrition and health.

53 The hormone-induced transcription factor E93 regulates temporal specific gene expression by controlling DNA regulatory element accessibility Chris Uyehara, Spencer Nystrom, Mary Leatham-Jensen, *Daniel McKay*. Departments of Biology and Genetics, Integrative Program for Biological and Genome Sciences, The University of North Carolina at Chapel Hill, Chapel Hill, NC.

Specification of cell identities during development requires precise regulation of gene expression in both space and time. While much work has focused on understanding how transcription factors control spatial specific gene expression programs, the mechanisms underlying temporal specific gene expression are less well understood. To address this gap, we have focused on the *cis*-regulatory control underlying elaboration of the *Drosophila* wing over time. Previous work from our lab revealed that *cis*-regulatory elements such as transcriptional enhancers can be identified with high accuracy through genome-wide open chromatin profiling. Using this approach, we found that the thoracic appendages (legs, wings, and halteres) share nearly identical open chromatin profiles despite exhibiting strikingly different morphologies. We also found that the appendage open chromatin profiles changed over developmental time. Interestingly, this change was coordinated between the appendages, suggesting the involvement of a systemic signal such as the steroid hormone ecdysone. To test this hypothesis, we performed an open chromatin profiling time course on wings from flies mutant for the ecdysone-induced transcription factor E93. Relative to wild type wings, E93 loss of function mutants exhibit global defects in open chromatin. However, these defects are almost entirely restricted to open chromatin sites that are temporally dynamic in wild type wings; only a small fraction of temporally static open chromatin sites are affected in E93 mutants. ChIP-seq reveals that most of the affected sites are directly bound by E93 *in vivo*. Transgenic reporter analyses also demonstrate that these sites are temporally specific transcriptional enhancers. Together, these data indicate that the ecdysone-induced transcription factor E93 controls temporal specific gene expression by controlling accessibility of temporal specific enhancers genome-wide.

54 Properties of enhancer RNA during embryonic development *Olga Mikhaylichenko*, Vladyslav Bondarenko, Dermott Harnett, Ignacio Schor, Eileen Furlong. Genome Biology, EMBL, Heidelberg, Germany.

Recent studies indicate that enhancers recruit RNA polymerase II (Pol II) and are transcribed into unstable non-coding RNA termed enhancer RNA (eRNA), adding a new layer of complexity to gene regulation. Both the timing of Pol II recruitment to enhancers, and eRNA production, appear tightly correlated with the timing of enhancer activity. However, the functional role of eRNAs, if any, is still actively debated and includes models for a function of the non-coding eRNA itself, or for the process of transcription i.e. an active transcriptional bubble at the enhancer. At the other extreme, some argue that eRNAs may simply represent transcriptional noise resulting from random engagement of Pol II to open chromatin regions. Regardless of the mechanism, these studies raise the question of the relationship between promoter and enhancer function, an issue we tackle in this study. Although extensively studied in mammals, enhancer transcription remains largely unexplored in *Drosophila*. Using *Drosophila* as a model provides access to a unique set of ~5,000 developmental enhancers whose spatial and temporal activity has been characterized in transgenic embryos. To initiate this study, we performed tissue-specific ChIP-seq on initiating and elongating forms of Pol II during embryonic mesoderm and nervous system development. We complement this with diverse types of RNA-seq, such as strand specific ribo-depleted RNA-seq and 5'CAGE. Considering the density of *Drosophila* genome, we show that many enhancers are associated with active transcription as a consequence of their genomic location, are the result of transcriptional read through, or are possible alternative transcription start sites. To disentangle RNA running through enhancers from true eRNA initiating from enhancers, we performed strand-specific PRO-cap, which maps nascent transcripts to their initiation sites. Our results provide the first comprehensive map of eRNAs during *Drosophila* embryogenesis, and demonstrate that *Drosophila* eRNA has similar properties to mammals. Using the large collection of characterised developmental enhancers, we show that the association between eRNA and activity is weaker than studies in mammals suggest and provides limited additional predictive power over DHS for activity. Using the PRO-cap signal we divide enhancer eRNA into two classes, those with focused, directional initiation, and those where initiation is more dispersed, and assess the ability of both types to function as an enhancer or a promoter in transgenic embryos. Our results demonstrate that a subset of developmental enhancers can also act as promoters in vivo, and uncovers a complex relationship between both functions which will be presented in detail.

55 Redundant GA-binding early transcription factors regulate the *Drosophila* histone locus body *Leila Rieder*[1], Kara Boltz[2], Guray Kuzu[3], Katy Curry[2], Jennifer Urban[1], Michael Tolstrukov[3], Robert Duronio[2], Erica Larschan[1]. 1) Molecular Biology, Cellular Biology and Biochemistry, Brown University, Providence, RI; 2) Biology Department, University of North Carolina-Chapel Hill; 3) Genetics Department, Harvard University, Cambridge MA.

Within the complex environment of the nucleus, specific transcriptional and regulatory processes are coordinated within membraneless domains known as nuclear bodies. These bodies, which are critical for the precise spatial and temporal regulation of the genome, include the conserved histone locus body (HLB), which regulates the cell cycle transcription and unique processing of the core histone genes. The histone genes are arrayed in ~100 tandem copies

at the histone locus, around which the HLB forms during embryonic development. While multiple factors are known to recruit to the HLB, how the body is developmentally established remains unknown. Using classical genetic and microscopy techniques, we sought to determine the genetic *cis* elements and protein factors required to initiate HLB formation. Here we show that the conserved GA-repeats in the 300bp Histone3-Histone4 promoter are required for HLB factor recruitment in *Drosophila*. Two known transcription factors, Chromatin Linked Adaptor for MSL Complex (CLAMP) and GAGA Factor (GAF), bind GA-repeats, but immunostaining in multiple tissues suggests that only CLAMP is present at the HLB in wild-type situations. ChIP-seq experiments reveal that CLAMP specifically recruits to the GA-repeats in the H3-H4p and regulates local chromatin accessibility and histone gene transcription, while GAF is specifically excluded. Animals lacking CLAMP expression die at the larval stages, but immunostaining revealed that these animals still possess active HLBs, and a small amount of maternally deposited CLAMP remains bound at the HLB. Further, in CLAMP depleted tissues, GAF re-localizes to the HLB, suggesting redundancy of transcription factor function. We conclude that GA-repeat binding factors are required for HLB formation in *Drosophila*.

56 Quantitative and predictive models of *even skipped* and *rhomboid* enhancers targeted by engineered transcription factors in the early *Drosophila* embryo *Garth Ilsley*[1], Justin Crocker[2], David Stern[2]. 1) Okinawa Institute of Science and Technology Graduate University, Onna, Japan; 2) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, USA.

Reasoning about fly regulation and developmental patterning generally requires simple, easily digestible models, typified by those commonly found in gene regulatory diagrams showing known activators and repressors. These models are useful for summarising the results of single input perturbations, but they come with three assumptions that make them difficult to apply to quantitative data and to lower-level regulatory features. The three limiting assumptions are that each regulatory input is either a simple activator or repressor, that each input has an independent threshold (giving rise to an ON or OFF input), and that regulatory inputs are combined using binary logic (AND, OR). To address these limitations more complex models can be used that have multiple biophysical parameters for lower-level features such as transcription factor binding sites or enhancer sequence. However, the complexity of these models makes them difficult to reason with and, moreover, they are often poorly predictive of even single input perturbations.

The growing availability of accurate, quantitative data at single-cell resolution makes an intermediate approach more broadly applicable. Ilsley et al (2013) showed that a simple sequence-free regulatory input function, trained using such data, is able to accurately predict perturbations of *trans* factors, including null mutants and ectopic expression of transcription factors that bind to the *eve* stripe 2 and *eve* stripe 3+7 enhancers. More recently, we extended this approach (Crocker et al, 2016) to model the acquisition of new regulatory activity at enhancers, using models for the *eve* stripe 3+7, *eve* stripe 4+6 and *rhomboid* enhancers. Instead of modifying enhancer sequences, we employed engineered transcription activator–like proteins fused to activators or repressors to target new transcription factor activity to enhancers. In each case we tested, we found that additional regulatory input combined linearly with the existing inputs and that our approach accurately modelled each enhancer's transcriptional output. Without explicit biophysical parameters, the model results can be related directly to hypotheses regarding loss and gain of binding sites and to lower-level features such as cooperativity and concentration-dependent transitions from activation to repression for specific transcription factors. Furthermore, these models enable the predictable 'tuning' of enhancers, providing a framework for the quantitative control of enhancers with engineered transcription factors.

57 Histones Abundance Adjusts the Timing of the Mid-Blastula Transition in *Drosophila* Henry Wilky, *Amanda Amodeo*. Lewis-Sigler Institute, Princeton University, Princeton, NJ.

The mid-blastula transition (MBT) is a crucial period during embryogenesis where the embryos of many species including flies, fish, and frogs switch from fast cell cycles driven by maternal components to slower cycles dependent on zygotic transcripts. In *Drosophila* the MBT is concurrent with cellularization of the syncytium that defines the early nuclear decisions. The timing of the MBT is sensitive to changes in the ratio of DNA to cytoplasm which is exponentially increasing as cells complete repeated rounds of DNA replication without growth. This lead to the hypothesis that titration of a maternally provided component against the increasing quantity of DNA may serve as a trigger to activate many downstream events. Our work suggests that histones may be this titrated component. We show that in *Drosophila* quantitative depletion of the early embryonic histone pool leads to early cell cycle slowing and premature gastrulation. Similarly, histone depleted embryos activate transcription of many early genes more strongly and in earlier cell cycles than controls. Our work demonstrates that alteration of histone levels can modify the timing of the MBT in *Drosophila* and opens the door for more detailed molecular analysis of the interaction between histone levels, transcription factors, and the cell cycle machinery.

58 Zelda binding sites as quantitative regulators of target gene transcription *C. Rushlow*[1], S. Yamada[1], P. Whitney[1], E. Eck[2], H. Garcia[2]. 1) Dept Biol, 1009 Silver Center, New York Univ, New York, NY; 2) Dept of Molecular

& Cell Biology and Dept of Physics, University of California at Berkeley, Berkeley, CA.
Zelda (Zld) is a ubiquitous transcriptional activator that potentiates the activity of the patterning morphogens during early embryonic development (Nien et al., 2011). For example, Zld is required for the proper spatiotemporal expression of the Dorsal (Dl) target gene *sog*, which is expressed in the lateral region of the blastoderm embryo where Dl levels decline. The *sog* shadow enhancer contains four strong Dl binding sites and three strong Zld binding sites. Removing Zld sites caused the expression domain to narrow; the more sites removed, the narrower the domain (Foo et al., 2014). To examine if and how Zld binding sites function as quantitative regulators of transcription, we monitored the dynamics of transcription in real time across successive nuclear cycles. We fused the *sog* gene enhancer, with or without Zld binding sites, to a reporter gene containing 24 MS2 stem loops upstream of the *yellow* (*y*) reporter gene. Transgenic males carrying these constructs are mated to females expressing EGFP-tagged MCP (MS2 coat protein) maternally. As the MS2 stem-loop RNAs are synthesized, MCP is recruited to the stem loops, and a local enrichment of signal appears as a distinct spot in the nucleus. We are measuring several aspects of the transcriptional dynamics by live imaging, for example, the time at which signal appears after mitosis in successive mitotic cycles, the amount of signal in each nucleus, and whether the timing and/or amount changes across the expression domain (ventral versus dorsal regions). Our preliminary results indicate that in the absence of Zld sites, not only is transcriptional activation delayed a mitotic cycle, it is delayed in subsequent cycles, indicating that Zld must continue to play a potentiating role in each cycle. In addition, nuclei within the domain of expression are less likely to activate transcription simultaneously, and some nuclei do not initiate transcription at all and are less like to give rise to transcribing daughter nuclei in the next cycle. We are currently addressing the question of how transcriptional dynamics changes across the dorsoventral axis in order to determine how Zld, which is uniformly distributed, interacts with the Dl gradient.

59 Intra-nuclear concentration and DNA-binding kinetics of Zelda defines zygotic genome activation in Drosophila *Dimitrios Papadopoulos*, Pavel Tomancak. Max-Planck Institute of Cell Biology and Genetics, Dresden, Saxony, Germany.
During early embryogenesis, maternally deposited messanger RNAs are progressively degraded, as the zygotic genome becomes primed for initiation of transcription. The transcriptional regulator Zelda (Zld) has been identified as a major player in rendering the genome receptive to transcription. However, while Zld is present in the syncytium from the first nuclear division onwards, activation of the zygotic genome does not occur until precisely 13 nuclear divisions later, indicating that it is subject to strict temporal control.
We have undertaken a quantitative approach to understand the kinetics of zygotic genome activation. Using Fluorscence Correlation Spectroscopy we have observed changes in the intra-nuclear concentration, distribution and DNA-binding kinetics of Zld and have quantified its mobility and variation of concentration among nuclei, before, during and after the timepoint of zygotic genome acitvation. We argue that the concentration of Zld determines its DNA-binding kinetics and therefore the onset of zygotic gene expression. In ongoing experiments we are inquiring how perturbations in concentration may confer predictable outcomes as to the timing of zygotic genome activation.
Our results underline the importance of Zld (and generally transcription factor) concentration and kinetics in instructing developmental fates. They also suggest that changes in transcription factor abundance and mobility may contribute to the tight temporal control of zygotic genome activation.

60 Live cell imaging of secondary cells reveals the subcellular dynamics of secretory and endosomal compartment formation and maturation *B. Kroeger*, F. Castellanos, C. Wilson. Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK, OX1 3QX.
Dissecting out the mechanisms that control the generation and fate of different secretory and endosomal compartments is not only of fundamental biological importance, but also impacts on our understanding of diseases such as cancer, diabetes and neurodegenerative disorders. We study a highly secretory epithelial cell type found in the male *Drosophila* accessory glands, the secondary cell (SC). SCs contain numerous large (3-10 μm diameter) subcellular compartments of different identities, which can be defined by assessing acidity and their association with specific Rab family GTPases. These compartments include acidic Rab7-positive late endosomal MVBs and lysosomes (MVBLs), and Rab11-positive non-acidic compartments, many of which, like other secretory cells, assemble secreted proteins in dense core granules (DCGs). Work in the lab has shown that secondary cells secrete nanovesicles called exosomes and DCG proteins into seminal fluid, which are then transferred to the female upon mating to modulate her behaviour. Using a combination of molecular genetic approaches and recently developed methods of *ex vivo* live cell imaging, we show how the different types of subcellular compartment form and mature in SCs. Firstly, we describe how fusion events between small acidic vesicles and non-acidic compartments are involved in the biogenesis of large MVBLs. We show a novel role for proton pump-carrying intraluminal vesicles in the acidification of these compartments. In addition, we find that dynamic fusion events lead to the initial formation of large non-acidic compartments at the trans-Golgi network. These compartments then undergo dramatic positional

and morphological re-arrangements, as proteins are trafficked into them and DCGs assemble. These processes are accompanied by changes in Rab protein association. Genetic analysis of Rab functions demonstrate they are essential for these maturation processes and in maintaining the normal balance of endocytic and secretory compartments. We are currently analysing other potential regulators of these subcellular events. By overcoming the technical limitations that previously prevented long-term live cell imaging of SCs, this work sheds new light on the dynamic processes that underpin basic cell biological decisions that drive secretory events. Our study implicates key roles for specific Rab GTPases. It also reveals mechanisms whereby the formation and identity of large intracellular compartments are determined by dynamic interactions with other, smaller compartments. Identifying the genetic programmes that control these interactions should provide new leads in our understanding of diseases affecting secretion and endosomal processing.

61 Imaging Hedgehog, Patched and Smoothened during signal transduction *Ryo Hatori*, Weitao Chen, Thomas Kornberg. University of California San Francisco, San Francisco, CA.

Many fascinating questions remain to understand how Hedgehog (Hh) travels from its site of synthesis to its target cell, and how a target cell transduces the Hh signal. We found that the larval air sac primordium (ASP), which develops in close association with the wing imaginal disc and is dependent on Dpp and Bnl(FGF) produced by the wing disc, also requires Hh. We observed that ASP development is affected by genetic conditions that either increase or decrease the level of Hh signaling in the ASP, and that Hh signal transduction in the ASP is graded such that cells closest to the Hh-producing disc cells have the highest levels of Hh signaling. Using BAC transgenes that express Hh, Patched (Ptc), or Smoothened (Smo) fused to fluorescent proteins (FPs), we studied how Hh:GFP, Ptc:GFP, Ptc:mCherry, Smo:GFP, and Smo:mCherry distribute in the ASP cells. We observed that Hh:GFP produced in the wing disc travels to the ASP via cytonemes that extend from the ASP and that vesicles with either Ptc:FP or Smo:FP traffic along the cytonemes. We also documented the distinct and graded subcellular locations of the Hh, Ptc and Smo FPs in the ASP cells, and noted that the distribution of Smo:GFP fluorescence was different from that of Smo:mCherry fluorescence. We attribute the different distributions of Smo:GFP and Smo:mCherry fluorescence to the more rapid maturation of the GFP fluorophore and to the pH sensitivity of GFP fluorescence. Smo:GFP fluorescence was mostly in the basal-lateral region was not observed more apically than the septate junction. Cells with higher levels of Hh signaling had more basal-lateral Smo:GFP. In contrast, Smo:mCherry preferentially localized to the sub-apical region of the cell and co-localized with markers for degradation endosomes. These observations are consistent with the idea that Smo is activated at the basolateral membrane and moves in the ASP cell to a sub-apical location where it is degraded. Together with the distinct distributions of Hh:FP and Ptc:FP-containing vesicles, these findings provide new perspectives on the process of Hh signal transduction.

62 Adenosine receptor signalling contributes to Grindelwald-induced JNK signalling in *scribbled* mutant tissue *Ingrid Poernbacher*, Jean-Paul Vincent. The Francis Crick Institute, London, United Kingdom.

JNK signalling is activated in *scribbled* mutant clones that are surrounded by wild-type cells in wing imaginal discs. As a result, these clones are eliminated by a process akin to cell competition whereby cells would compare their polarity status. However, wing discs from wholly *scribbled* mutant larvae also upregulate the JNK pathway. Likewise, JNK signalling is activated throughout large domains of *scribbled* deficient cells, not only at the edges. This suggests that the loss of *scribbled* could activate JNK signalling through cell competition-independent mechanisms. We found that this mode of JNK activation is substantially impaired in larvae lacking the *Adenosine receptor* (*AdoR*) while loss of this receptor has only a mild impact on JNK activation in *scribbled* clones. A similar effect is seen upon overexpression of intracellular Adenosine deaminase (Ada), which converts intracellular Adenosine to Inosine, or by loss of the *equilibrative Adenosine transporter Ent2*. This suggests that loss of apico-basal polarity causes the release of intracellular Adenosine and subsequent activation of the AdoR, which in turn increases JNK pathway activity. Importantly, removal of the *AdoR* also reduces JNK activation caused by overexpression of an active form of Grindelwald, a TNF receptor that generally mediates JNK activation in polarity-deficient tissue including *scribbled* clones. We conclude that Adenosine receptor signalling modulates this Grindelwald-dependent activation of the JNK pathway during the chronic widespread loss of *scribbled.*

63 Regulation of Dpp signaling by O-linked glycosylation *Matthew Moulton*[1], Alexander Kim[1], Gregory Humphreys[2], Anthea Letsou[1]. 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) Pennsylvania State University, University Park, PA.

Animal embryogenesis requires input from diverse signaling pathways to coordinate proper placement and organization of body structures, tissues, and organs. Activation and deactivation of signaling pathways at the right time and place is essential for embryogenesis, with defects in signaling often leading to inborn errors of development. A molecular and biochemical understanding of signal transduction pathways is important for proper

diagnosis and treatment of congenital developmental abnormalities. Our lab uses the fruit fly, *Drosophila melanogaster*, to understand developmental defects caused by alterations in Decapentaplegic (Dpp) signaling. Dpp is a homologue of vertebrate BMP2/4 and is a member of the TGF-β family of cytokines. In the fly, both losses and gains of Dpp signaling result in embryonic lethality with associated defects in dorso-ventral patterns and structures.

While we have previously reported that loss of the *mummy* (*mmy*)-encoded UDP-*N*-acetylglucosamine diphosphorylase results in ectopic Dpp signaling and embryonic lethality, here we provide mechanistic insight into the specific role of GlcNAc in Dpp signal antagonism. Our studies have their foundation in our discovery of *super sex combs* (*sxc*), the Drosophila O-GlcNAc transferase, in an RNAi screen for glycosyltransferases that share loss-of-function phenotypes with *mmy*. Here we show that, like Mmy, Sxc is a Dpp signaling antagonist that functions to restrict Dpp signal transduction in the *Drosophila* embryonic epidermis. Loss of *sxc* results in ectopic Dpp phenotypes, including an expanded pMad domain and a loss of larval ventral denticle belts. Notably, these markers of ectopic Dpp signaling persist in the absence of the Dpp Type I receptor Thickveins (Tkv), but require Saxophone (Sax), which we demonstrate is O-link glycosylated in vivo by Sxc. Taken together, our data point to Sax as a potent Dpp signal transducer in the embryonic epidermis, with the capacity to activate Mad well beyond the domain normally specified by Tkv. We speculate that epidermal Tkv and Sax function as two Dpp receptors with differential signaling range potential, and that Sxc-mediated inhibition of Sax limits epidermal Dpp signaling exclusively to Tkv. Our studies are the first to: 1) demonstrate a role for Sxc in embryogenesis, and 2) provide mechanistic insight into the role of GlcNAc in modulating responses to Dpp.

64 VEGFR/Pvr signaling regulates diverse responses during wound healing in *Drosophila* larvae *Chang-Ru Tsai*[1,2], Sirisha Burra[2], Michael Galko[1,2]. 1) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Department of Genetics, MD Anderson Cancer Center, Houston, TX.

To cope with inevitable injury, organisms possess efficient wound healing mechanisms that maintain tissue integrity. However, the genetic details by which epidermal repair is accomplished remain poorly defined. *Drosophila* larval epidermis shares similar wound healing dogma as mammals. Our lab identified three responses during *Drosophila* larval epidermal wound healing: epidermal cell migration to close the wound gap, blood cell (hemocyte) attachment at the wound site, and hemocyte spreading from a spherical shape to a fan-like appearance.

Previously, our lab found that the *Drosophila* homolog of the Vascular Endothelial Growth Factor (VEGF) receptor, a receptor tyrosine kinase (RTK), and its ligand, Pvf1, are required for epidermal wound closure. Since Pvr is also highly expressed on hemocyte membranes, we tested if Pvr is also required for hemocyte spreading. Indeed, purified Pvf1 ligand is able to induce hemocyte spreading *in vitro*. Overexpression of a constitutively active form of the Pvr in the hemocyte also leads to enhanced hemocyte spreading. Consistent with these expression and gain-of-function results, loss-of-function of Pvf1 or Pvr caused decreased actin polymerization and hemocyte spreading at wound sites *in vivo*, indicating that Pvr signaling also regulates hemocyte spreading during normal wound physiology. Therefore, a single RTK signaling pathway regulates both epidermal wound closure and hemocyte spreading. We next asked whether the same or different downstream mediators are employed in each response. Using a novel genetic screening platform, I identified 31 genes as potential Pvr downstream effectors. I next tested their roles in both hemocyte spreading and epidermal wound closure to define the precise signaling architecture in each Pvr-dependent response. Our results show that there are common and unique downstream effectors in each context. Given that VEGF/Pvr signaling plays a critical role during tissue repair, identification and analysis of the genes studied here is likely to have significant implications for enhancing our understanding of wound healing in humans.

65 Rho family GTPases respond to pattern established by RhoGEFs in cell wound repair *Mitsutoshi Nakamura*, Susan M. Parkhurst. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Rho family GTPases are molecular switches and key regulators for actin and myosin reorganization in many biological processes including cell migration, cytokinesis, and wound repair. To reorganize actin and myosin, the activities and localization of Rho family GTPases are regulated spatially and temporally. We are using single cell wound repair as a model for examining this spatial/temporal regulation of Rho family GTPases. When cells are wounded, Rho family GTPases accumulate around wounds and exhibit discrete localization patterns, which contribute to the accumulation of actin and myosin II at the wound edge and their formation into an actomyosin ring. To investigate the mechanisms governing Rho family GTPase specific localizations upon wounding, we are focusing on the role of Rho GEFs (Rho guanine nucleotide exchange factors) that are known to be major upstream regulators of these GTPases. To date, we have tested 12 out of the 26 known *Drosophila* RhoGEFs and found that three of these - RhoGEF2, Pebble (Pbl), and RhoGEF3 - are recruited to wounds and required for cell wound repair. This result was surprising as RhoGEF2 and Pbl functions have so far been mutually exclusive (i.e, RhoGEF2 is a component of the maternally-required cytokinesis machinery, whereas Pbl is a component of the zygotic cytokinesis machinery). Interestingly, we find that the three RhoGEFs are recruited to cell wounds in discrete localization patterns

similar to those exhibited by Rho1, Rac, and Cdc42, suggesting that Rho family GTPases are simply responding to patterns already established by the RhoGEFs rather than creating these patterns. In addition, we examined crosstalk among the three RhoGEFs, Rho family GTPases, and the cytoskeleton by genetic and pharmacological assays. We found that crosstalk among RhoGEFs and the cytoskeleton are required for RhoGEF recruitment to wounds.

66 The Tip60/Enhancer of Polycomb (E(Pc)) complex is a tumor suppressor that represses hematopoietic tumors by negatively regulating JAK/STAT signaling *Alessandro Bailetti*, Abigail Anderson, Lenny Negron-Piñeiro, Vishal Dhruva, Erika Bach. Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY.

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that cause excessive proliferation of specific myeloid lineages. The majority of MPN patients have a point mutation in the *JAK2* gene ($JAK2^{V617F}$), which generates a constitutively-active kinase that hyperactivates the JAK/STAT pathway. In *Drosophila,* JAK/STAT signaling is conserved but simplified, with a single JAK Hopscotch (Hop) and a single STAT (Stat92E). One *hop* allele - hop^{Tum-l} - is dominant and hyperactivates the JAK/STAT pathway. Like MPN patients, hop^{Tum-l} mutants have dramatically increased numbers of myeloid cells, which are plasmatocytes and lamellocytes that aggregate to form melanotic tumors and frequently kill the host. The hop^{Tum-l} tumor phenotype is dependent on JAK/STAT signaling, and reducing one copy of *Stat92E* strongly suppresses the tumor burden. To identify genes that dominantly modify the hop^{Tum-l} phenotype, we performed a deficiency screen and identified 11 enhancers and 8 suppressors.
A deficiency that uncovers *Enhancer of Polycomb (E(Pc))* significantly enhanced melanotic tumor burden and lethality in hop^{Tum-l} animals. This enhancement was recapitulated by heterozygosity for *E(Pc)* alleles. To test if *E(Pc)* has a role in blood development, we depleted *E(Pc)* from hematopoietic tissue. Hematopoietic depletion of *E(Pc)* caused the differentiation of lamellocytes; by contrast, this cell type was absent from control animals. Additionally, larval hemolymph bleeds of *E(Pc)*-depleted animals revealed small melanotic tumors, which were never observed in control larvae. E(Pc) is a highly conserved protein that co-purifies with the Tip60 Histone Acetyl Transferase complex. Depletion of *Tip60* or of most complex components, including *Bap55* and *Domino*, resulted in lamellocyte differentiation, consistent with the model that E(Pc) functions through the Tip60 complex in blood cells.
To determine the mechanism by which E(Pc) represses melanotic tumors, we tested whether the *E(Pc)* tumor phenotype was dependent on JAK/STAT pathway activity. We found that *Stat92E* depletion blocked tumor formation upon *E(Pc)* knock down, suggesting that E(Pc) is upstream of Stat92E. Consistent with this, we showed that *E(Pc)* mutant clones have increased JAK/STAT signaling and that *Tip60*-depleted hemocytes have increased expression of JAK/STAT target genes (*Socs36E* and *domeless*). These data strongly suggest that E(Pc) represses lamellocyte differentiation by attenuating JAK/STAT activity, possibly by either direct acetylation of Stat92E or by transcriptional induction of suppressors of Stat92E.

67 Gene regulatory networks evolve at different nodes in different developmental contexts *Sebastian Kittelmann*[1], Alexandra D. Buffry[1], Georgina Haines-Woodhouse[1], Isabel Almudi[2], Saad Arif[1], Nico Posnien[3], José Luis Gómez-Skarmeta[2], Alistair P. McGregor[1]. 1) Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; 2) Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Sevilla, Spain; 3) Department of Developmental Biology, Georg-August-University Göttingen, Germany.

Changes in gene regulatory networks (GRNs) underlie the evolution of morphology. However, it is unclear if the nodes at which a GRN can evolve are different when phenotypic features are lost versus gained and how developmental context influences evolvability. We have used the GRN underlying trichome development In *Drosophila* to approach these questions. It was previously shown that larval trichomes have convergently been lost in several *Drosophila* species due to changes in *cis*-regulatory modules (CRMs) of *ovo/shavenbaby* (*svb*), which is both necessary and sufficient for trichome formation. Loss of *svb* expression thus results in loss of trichomes. Furthermore, this gene has been proposed to act as an input-output device by integrating upstream patterning information and directing transcription of a battery of downstream factors. Trichome patterns on *Drosophila* legs have also evolved between and within species, but here the gain of trichomes is linked to changes in the expression of the HOX gene *Ultrabithorax* and the micro-RNA gene *miR-92a*, respectively. To explore why the trichome GRN might evolve at different nodes in different tissues and when trichomes are gained rather than lost, we have characterised the leg trichome GRN. We used RNA-seq to identify the genes from the larval GRN that are also expressed during leg trichome formation and ATAC-seq to identify the CRMs of key genes. We subsequently tested if specific genetic interactions are direct or indirect. Our findings suggest that the architecture of GRNs differs depending on the developmental context. This can influence which nodes evolve and helps to determine whether phenotypic features can be gained or lost.

68 Interaction Of *Cis*-Regulatory Changes At Two Loci In The Evolution Of The *Drosophila prolongata* Sensory System. *David Luecke*, Artryom Kopp. Evolution and Ecology Dept, University of California - Davis, Davis, CA.

Gene expression evolution is a major contributor to phenotype diversification, yet the complexity of transcriptional networks often leaves the molecular mechanisms unclear. Instances of rapid phenotypic evolution with clear candidates for causal gene expression evolution are powerful tools to gain leverage on this problem. A massive increase in the number of chemosensory bristles on the male foreleg of *D. prolongata* is not seen in any other related species, including sister species *D. rhopaloa*. Antibody staining shows expansion of expression of doublesex (dsx) and Pox neuro (Poxn), the genes responsible for sexually dimorphic development and chemosensory bristle fate respectively, into cells developing as the novel chemosensory organs. *Cis*-regulatory elements (CREs) from each of these loci, from both the *D. prolongata* and *D. rhopaloa* genomes, were cloned into a reporter construct and examined in a *D. melanogaster* transgenic background. The dsx CRE from *D. prolongata* produces expression in much of the leg region homologous to the novel chemosensory bristles, which is not seen from the homologous CRE of *D. rhopaloa*. The *D. prolongata* Poxn CRE shows a similar expansion, only into a smaller subset of the leg region. These results indicate most of the doublesex expression evolution can be explained by changes in *cis* to the locus, while evolution of Poxn expression involves a combination of changes in *cis* and in *trans* to the Poxn locus. To account for sensitivity of the Poxn CRE to trans-level changes in dsx expression, composite genotypes were constructed that expand dsx expression to mimic that produced by the evolved dsx CRE, and show the expression given by the Poxn CRE in the modified transcriptional background. These genotypes can be produced with either *D. rhopaloa* or *D. prolongata* sequence from either locus, which enables discrimination between additive and epistatic interaction models. This approach is a novel way to use the power of *D. melanogaster* transgenic tools to discover the role gene expression interactions play in producing naturally occurring developmental variation.

69 Conservation and evolution of maternally deposited and zygotically transcribed mRNAs in the early Drosophila embryo Joel Atallah[1,2], *Susan E. Lott*[1]. 1) University of New Orleans, New Orleans, LA; 2) Evolution and Ecology, University of California, Davis, Davis, CA.

In all animals, early development is facilitated by maternally deposited mRNAs and proteins. Maternal transcripts are degraded as the zygotic genome is transcriptionally activated, in a highly regulated process known as the maternal to zygotic transition (MZT). While this process has been well-studied in model species, we have only limited knowledge of natural variation or evolution of the transcripts present at these critical stages of development. Here, we created a transcriptomic dataset for 14 *Drosophila* species, at developmental stages before and after the MZT, and compared our results with previously published data on the developmental transcriptome of *Aedes aegypti*. We studied stage-specific changes in transcript representation at each stage on the species phylogeny. Broadly, there is remarkably high conservation of transcript levels across early embryogenesis, especially for those genes whose transcripts are both maternally deposited and zygotically transcribed. We found hundreds of genes where the maternally deposited isoforms differ from zygotically transcribed isoforms, with the exonic structures of these stage-specific transcripts frequently being conserved over tens of millions of years of evolution. While maternally deposited transcripts are generally highly conserved compared to purely zygotic genes, the subset of maternal transcripts which are completely degraded at the MZT varies dramatically between species. While generally zygotic transcripts vary more than maternally deposited transcripts, we found a small group of purely zygotic genes that are extremely conserved across hundreds of millions of years of evolution. This select group is highly enriched in transcription factors that play critical roles in early development. The transcripts present at both the maternal and zygotic stages are generally under the highest level of developmental constraint, while those expressed by only the maternal or zygotic genome evolve more freely. Phylogenetic analysis of changes in transcript representation at each stage shows accelerated rates of evolution in specific lineages, including the *obscura* species group. This study opens avenues for future research investigating whether clade-specific changes in maternal deposition or zygotic transcription are associated with particular life-histories or ecologies.

70 Reverse-engineering the evolution of Drosophila mesoderm invagination Silvia Urbansky, Paula Gonzalez, Viola Noeske, Francesca Caroti, *Steffen Lemke*. Centre for Organismal Studies, Universitaet Heidelberg, Heidelberg, Germany.

The diversity of animal form is linked to developmental differences in morphogenesis, i.e. the coordinated and shape-giving behavior of cells and tissues. Understanding the evolutionary transition between distinct programs of morphogenesis requires a precise description in cell and tissue behavior, the identification of changes in gene activity, and a functional validation to support the link between molecular and morphogenetic divergence. To understand and functionally test the evolution of morphogenetic programs, we use early embryonic development in *Drosophila melanogaster* as reference and compare its highly optimized and efficient process of germ-layer formation with gastrulation in other fly species.

During gastrulation of *D.melanogaster*, mesoderm internalization along the ventral midline starts from a uniform and columnar epithelium which invaginates as a coherent epithelium through tight coordination of cell behavior. In the midge *Chironomus riparius*, as well as other insects representing presumably more ancestral modes of insect gastrulation, mesoderm internalization starts with a cuboidal epithelium from which cells ingress individually and in a

stochastic manner. We have identified two genes, *folded gastrulation* and *t48*, which in the evolution of fly gastrulation acted as a likely switch from the ingression of individual mesoderm cells to the invagination of the blastoderm epithelium. Both genes play a critical role in mesoderm invagination *D.melanogaster*, but they do not appear during mesoderm ingression of *C.riparius*. We could demonstrate that early expression of these genes in *C.riparius* is sufficient to invoke mesoderm invagination similar to *D.melanogaster*. 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 the mode and outcome of morphogenesis.

71 Stress-dependent miRNA-based regulation of Rbfox1/A2bp1 promotes RNP granule formation and cell survival Mariya Kucherenko, *Halyna Shcherbata*. Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

There is increasing evidence that regulation of gene expression in response to stress happens at the post-transcriptional level in the specialized subcellular membrane-less compartments (RNP granules). They assemble via concentration-dependent phase separation of LCD-containing RNA-binding proteins. These enigmatic proteins are one the largest challenges in modern biology. They are intrinsically disordered, which makes them impossible to crystalize; they can promiscuously interact with multiple proteins, which makes challenging to define their biological functions; and it has not been shown in the living organism what regulates their ability to be included in different RNPs to effectively regulate RNA metabolism upon reversible stress. Recently we discovered a novel compelling mechanism governing cellular concentration of one of these proteins, where a miRNA acts as a major regulator of its expression.

We found that Drosophila homolog of human Rbfox1 contains multiple LCDs. Depending on concentration and isoform composition, it can form various nuclear, cytoplasmic, and mitochondrial RNP granules, ranging from liquid droplets to hydrogels to beta-amyloid-like fibers. We uncovered an elegant mechanism by which Rbfox1 levels and isoform-specificity are adjusted by a stress-dependent miRNA. Reduced *miR-980* expression during stress leads to increased Rbfox1 levels, followed by widespread formation of RNP granules and changes in RNA metabolism and mitochondrial homeostasis, promoting cell survival.

Dysfunctions of human RBFOX proteins are associated with various medical conditions, including spinocerebellar ataxia type 2, mental retardation and epilepsy, attention-deficit hyperactivity disorder, autism, hand osteoarthritis, congenital heart defects, obesity and diabetes. We found that human RBFOX proteins also contain multiple LCDs and form membrane-less compartments, suggesting that the RNP-granule-linked control of cellular adaptive responses may contribute to a wide range of RBFOX-associated pathologies in humans.

We propose that miRNA-dependent regulation of phase-separating proteins may be a frequently used mechanism to regulate RNA metabolism in organisms exposed to environmental perturbations.

72 The N^6-methyladenosine (m^{6}A) RNA modification modulates neuronal functions and sex determination in *Drosophila melanogaster* *T. Lence*[1], J. Akhtar[1], M. Bayer[1], K. Schmid[2], L. Spindler[3], C. Hei Ho[4], N. Kreim[1], M.A. Andrade-Navarro[1], B. Poeck[3], M. Helm[2], J.-Y. Roignant[1]. 1) Institute of Molecular Biology, Mainz, Rheinland Pfalz, Germany; 2) Institute of Pharmacy and Biochemistry, Johannes Gutenberg University of Mainz, 55128 Mainz, Germany; 3) Institute of Zoology III (Neurobiology), Johannes Gutenberg University of Mainz, 55128 Mainz, Germany; 4) Kimmel Center for Biology and Medicine of the Skirball Institute, NYU School of Medicine, Department of Cell Biology, 540 First Avenue, New York, NY 10016.

N^6-methyladenosine RNA (m^{6}A) is one of the most abundant mRNA modifications in vertebrates. While its functions in the regulation of posttranscriptional gene expression are beginning to be unveiled, precise roles of this modification during development of complex organisms remain unclear.

We carried out a comprehensive characterization of the m^{6}A methyltransferase complex and YTH reader proteins in *Drosophila melanogaster*. Components of the complex show ubiquitous expression with clear enrichment in the nervous system, which is consistent with the high levels of m^{6}A in heads of adult flies. Using transcriptome wide m^{6}A profiling we show that modification is conserved, however some unique features distinguish *Drosophila* from vertebrates. Surprisingly, mutant flies for the catalytic subunits (Ime4 and dMettl14) are viable, but suffer from severe locomotion defects due to altered neuronal functions, which demonstrates the importance of m^{6}A in the *Drosophila* nervous system. Components of the m^{6}A methyltransferase complex also fine-tune the female-specific splicing of *Sex lethal* (*Sxl*) transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. Remarkably, knock out (KO) of the nuclear m^{6}A reader YT521-B resembles the loss of the catalytic subunits, implicating this protein as a main effector of m^{6}A functions *in vivo*. Lastly we identified a novel *bona fide* subunit of the methyltransferase complex, Spenito (Nito) that regulates m^{6}A levels and m^{6}A-dependent RNA processing.

Altogether, our study substantially extends the knowledge on m^{6}A biology, revealing the existence of novel components of the methyltransferase complex and demonstrates crucial roles for this RNA modification in

fundamental processes within the context of the whole animal.
Lence et al., Nature (2016, accepted)

73 A sex-specific small peptide is encoded by a large "ncRNA" within the *Drosophila* bithorax complex.. *C. Immarigeon*, F. Karch, R. Maeda. Geneva University, Genève, Genève, Switzerland.

Small polypeptides under 100 amino acids have long been discriminated against and excluded from genomic and proteomic studies. However, recent innovations in computing and experimental analyses (ribosome profiling and mass spectrometry) reveal that these small peptides (sPEPs) are actually abundant products of eukaryotic genomes. These sPEPs are encoded by small open reading frames (sORFs) present in every class of RNA-PolII transcripts in yeasts, plants, insects, and mammals. However, the contribution of these peptides to cellular functions remains largely unknown.

One sORF lies in the Bithorax complex (BX-C) of *Drosophila*, a well-known locus containing three homeotic genes (*Ubx, abd-A* and *Abd-B*) and their regulatory sequences. This sORF is part of a large transcript called *MSA* spanning most of the intergenic region between *abd-A* and *Abd-B*, and classified as non-coding. By CRISPR-guided gene conversion we knocked-in a GFP in frame with this sORF in the BX-C. Interestingly, we show that this sORF is translated into a peptide specifically in "secondary" cells of male accessory glands, the prostate-like organs of insects. These cells represent about 4% of the secretory cells of this organ, while the other 96% are "main" cells. We performed immunoprecipitation of ribosomes from secondary cells followed by RT-qPCR. We observe a strong enrichment for *MSA* and housekeeping mRNAs, and a depletion of main cell genes confirming the specificity of the technique and that *MSA* is undergoing translation. We generated a series of mutation in the sORF and tested their translation in cultured cells, revealing that several in-frame methionine codons can initiate translation, generating peptide isoforms of different length. We have also generated mutant flies knocked out for this sORF and we are in the process of probing the function of this peptide. We pay special attention to the so called post mating response in females that is mediated by the seminal fluid proteins secreted by the accessory gland. By coupling the powerful genetic tools offered by *Drosophila* to the wealth of knowledge about BX-C organization and function gained through more than 6 decades of research, we hope to find and document a function for this tiny peptide.

74 TDRD5P, a component of cytoplasmic processing body promotes male germline sexual identity *Caitlin Pozmanter*, Shekerah Primus, Mark Van Doren. Biology, Johns Hopkins University, Baltimore, MD.

The process of sex determination in *Drosophila* is regulated by the RNA-binding protein Sex lethal (SXL) in both the germline and the soma. While the mechanism by which SXL brings about sexual dimorphism in the soma is well studied, its function in the germline is less well understood. To identify genes that are regulated by SXL in the germline, RNA sequencing was done to compare *Sxl*-RNAi ovaries to control ovaries. This was done in a *bam* mutant background to enrich for undifferentiated, SXL-expressing, germline. Analysis of the expression data uncovered a previously uncharacterized gene CG15930 with a 16-fold increase in expression in *Sxl*-RNAi females, suggesting that SXL functions in the female germline to repress CG15930. CG15930 contains a single tudor domain and is most closely related to the mouse *TDRD5* and the Drosophila *TDRD5* homolog *tejas*, so we have named it *tdrd5-prime* (*tdrd5p*).

To elucidate the function of TDRD5P in the male germline, we generated mutant alleles using CRISPR/CAS9 along with an HA tagged genomic BAC construct for analysis of the TDRD5P protein. The *tdrd5p* mutant testes show several phenotypes such as germline loss, and a 50% reduction in fecundity indicative of an important role for TDRD5P in germline differentiation. Confocal microscopy of HA-TDRD5P gonads shows an interesting localization of TDRD5P to discrete cytoplasmic bodies. Co-staining for factors known to localize to cytoplasmic processing bodies (P-bodies), the sites of RNA degradation and repression, such as DCP1 and Me31b demonstrate that TDRD5P localizes to the P-body. Additionally, RNAi against the deadenylase *twin* in mutant gonads revealed a genetic interaction between *tdrd5p* and the CCR4-NOT deadenylation complex. RNA-seq to compare *tdrd5p* mutant testes to wildtype testes revealed a dramatic 500-fold increase in *mst36fb* expression in mutant testes. Interestingly, transcription of *mst36fb* is activated by meiotic arrest genes followed by translational repression of the mRNA through meiosis, suggesting a potential role for TDRD5P in regulating the progression of meiosis. Lastly, to determine which proteins TDRD5P interacts with, we are conducting co-immunoprecipitation followed by mass spectrometry. Taken together, our data suggests that the previously uncharacterized TDRD5P functions to ensure the proper development of the male germline and must be repressed by SXL to allow for female germline development.

75 A new aspect of the mid-blastula transition: regulation of histone/lipid droplet interactions controls histone levels in the nucleus *Matthew R Johnson*, Michael A Welte. University of Rochester, Rochester, NY.

Drosophila embryos contain hundreds of thousands of lipid droplets (LDs), fat storage organelles that provide a critical energy supply for embryogenesis. In early embryos, LDs are also associated with specific histones. It had

been proposed that this association buffers the histone supply: when histone cannot bind to LDs, histone H2Av over-accumulates in nuclei and embryos exhibit hallmarks of DNA damage and reduced viability. However, direct evidence for this buffering model has been lacking.

Using FRAP and photoactivation, we found that during cleavage stages H2Av spreads rapidly through the embryo, at rates similar to diffusion. This spread is not due to LD motion; rather, H2Av exchanges between LDs via the cytoplasm. This dynamic behavior of H2Av is developmentally regulated: In late oocytes and embryos up to cycle 13, H2Av exchanges rapidly between LDs. By 15 min into cycle 14, H2Av exchange between LDs ceases almost entirely and nuclear uptake of H2Av is decreased.

Together, these observations suggest two states of histone dynamics: Initially, H2Av is dynamically attached to LDs, rapidly exchanging between LDs via the cytoplasm; this exchange presumably buffers cytoplasmic H2Av levels and provides the source of histones for import into nuclei. After the transition, H2Av is statically attached to LDs; this abolishes buffering and limits delivery of H2Av into nuclei.

This striking change in histone dynamics occurs during the mid-blastula transition, when the zygotic genome undergoes profound remodeling, including massive upregulation of transcription and heterochromatin formation. We find that when histone sequestration to LDs is abolished, the heterochromatin protein HP1 accumulates prematurely in nuclei, suggesting that this system may regulate heterochromatin establishment. We are now analyzing the mechanistic basis for this switch in histone dynamics. It is not mediated by zygotic transcription or due to the massive intracellular redistribution of LDs which occurs around this time. Studies on modifications of the histone anchor protein Jabba and of the histones themselves are in progress.

Emerging evidence in multiple systems indicates that LDs have a general role in regulating nuclear functions, via the exchange of lipids, transcription factors, and chromatin components. Our work may provide a paradigm for how LDs control nuclear functions, via regulating and buffering protein availability.

76 Complementary molecular cues ensure a robust microtubule-dependent nuclear positioning in the *Drosophila* oocyte Nicolas Tissot[1], Jean-Antoine Lepesant[1], Fred Bernard[1], Kevin Legent[1], Floris Bosveld[2], Charlotte Martin[2], Orestis Faklaris[1], Yohanns Bellaïche[2], Maité Coppey[1], *Antoine Guichet*[1]. 1) Institut Jacques Monod, CNRS UMR 7592, Université Paris-Diderot, Sorbonne Paris Cité, 75205 Paris Cedex, France; 2) Polarity, Division and Morphogenesis Team, Institut Curie, CNRS UMR 3215, INSERM U934, 26 rue d'Ulm, 75248 Paris Cedex 05, France.

Controlling the localization of the nucleus is crucial for a variety of cellular functions. In the *Drosophila* oocyte, nuclear asymmetric positioning is essential for the reorganization of the microtubule (MT) arrays that control the polarized transport of axis determinants. Combining quantitative 3D live imaging and laser ablation-mediated force analysis reveals that nuclear positioning is ensured with an unexpected level of robustness. We show that the nucleus is pushed to the oocyte antero-dorsal cortex by MTs, and that its migration can proceed through distinct tracks. One migratory route is favoured by centrosome-associated MTs. Alternatively, a separate route is promoted by the MT-associated protein Mud/NuMA asymmetrically localized in a Asp-dependent manner at the nuclear envelope hemisphere where MT nucleation is higher. Our results demonstrate that centrosomes do not provide an obligatory driving force for nuclear movement, but together with Mud, contribute a set of complementary mechanisms that ensure the robustness of asymmetric nuclear positioning.

77 Global regulation of Pericentrin-Like-Protein transcript and protein controls its local positioning on the proximal end of centrioles *J. M Ortega*, K. M Plevock, T. A Schoborg, B. J Galletta, N. M Rusan. National Institutes of Health Bethesda, MD.

Pericentriolar material (PCM) is organized by centrioles to form centrosomes, the major microtubule-organizing center of cells. In *Drosophila melanogaster (D.mel)*, the giant centrioles of spermatocytes restrict the protein Pericentrin-Like Protein (PLP) to the proximal end. To investigate the role and mechanism of restricting PLP to the proximal end, we performed cross-species comparisons of fifteen *Drosophila* species. This evolutionary cell biology approach revealed both conservation and striking divergence from *D.mel*. Some species such as *D.ere* and *D.ana* restrict PLP proximally, others such as *D.vir* and D.*moj* exhibit PLP localization along the entire length of their centrioles. We focused on *D.moj* as the most extreme example of divergence. Using Structured Illumination Microscopy, we determine that PLP is fully instructive to PCM as it also localizes along the entire length of centrioles in *D.moj*. We next aimed to determine the mechanism that proximally positions PLP. EM studies revealed that the cartwheel, which is an internal proximal centriole structure, does not determine PLP localization. This contradicts a prominent hat the cartwheel determines PCM localization. We next tested the hypothesis that PLP is restricted to the proximal end by depleting the cytoplasmic PLP available during centriole distal end formation, or centriole elongation. Both tissue and single cell analysis of protein levels confirm that PLP is degraded in spermatocytes and is not available to load onto the centriole during its growth phase. To confirm this, we used the Gal4-UAS system to overexpress PLP in spermatocytes; this resulted in PLP localizing to the entire centriole length. Additionally, this was sufficient to guide PCM localization all along the centriole length. Using in situ hybridization, we

show that PLP RNA levels mimic the protein expression pattern in testes. Thus, we have shown that the proximal centriole zone where PCM is loaded is defined by the tight regulation of PLP RNA and protein. Furthermore, disrupting this pattern by forcing PLP along the entire centriole, results in basal body positioning defect, which appear to be a consequence of failed basal body docking to the nucleus. Interestingly, we show that *D.moj* does not regulate PLP RNA and protein likewise, indicating that other species have evolved alternate mechanisms to properly position and dock basal bodies.

78 Novel concepts of cytoskeleton regulation during neuronal growth, maintenance and degeneration *Yue Qu*, Ines Hahn, Meredith Lee, Jill Parkin, Andreas Prokop. Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, Manchester, United Kingdom.

The development of a functioning nervous system requires the formation of a proper network of axons, the up-to-a-meter-long cable-like cellular processes of neurons. These delicate structures have to be maintained for the life time of an organism, although they are vulnerable to damage through ageing, injury, and neurodegenerative diseases. The structural backbones and intracellular transport highways of axons are formed by parallel bundles of microtubules (MTs) which are surrounded by periodically spaced rings of cortical F-actin. Using *Drosophila* neurons in primary culture and *in vivo*, we decipher the machineries, composed of the different classes of MT- and actin-binding proteins, that regulate this cytoskeletal organisation in axons. From studies of ~50 such proteins we extracted the model of local axon homeostasis (Voelzmann *et al.*, 2016, *Brain Res Bulletin* 126, 226ff.). A central player is Short stop, a large actin-microtubule linker which guides the extension of polymerising MTs along cortical F-actin, thus laying axonal MTs out into parallel bundles. Here we propose two further novel mechanisms promoting the bundle organisation of axonal MTs: (1) A membrane-anchored cortical collapse factor serves as a check point by eliminating "off track" MTs that have escaped the guidance mechanism. When artificially detached from the membrane, this factor is able to deplete entire MT networks. In its absence, there is a gradual increase in "disorganised" MTs, correlating with nervous system decay. (2) Cortical actin stabilises MT polymerisation events, contributing to axon growth and maintenance. This mechanism was deduced from combining actin manipulations with readouts including (i) actin ring abundance upon super-resolution microscopy, (ii) axon morphology and (iii) the organisation and dynamics of axonal MTs.

Our findings provide new cell biological concepts for axons that can explain important aspects of their development, regeneration, degeneration and maintenance/ageing.

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79 Role of the formin Dia in formation of epithelial compartments *Anja Schmidt*, Zhiyi Lv, Jörg Großhans. Developmental Biochemistry, University Goettingen, Göttingen, Niedersachsen, Germany.

A characteristic feature of epithelial cells are their distinct compartments, i. e. apical, basal and lateral domains. Compartments are defined by the localization of distinct marker proteins: the apical domain by Crumbs, the lateral domain by Discs-large (Dlg), the basal domain by Integrin, for example. In addition, a dedicated subapical domain is formed at the boundary between apical and lateral compartment, containing the zonula adherens with adherens junction complexes, with Bazooka (Baz), Armadillo, and E-Cadherin (Shotgun), for example. The proper organization of membrane domains is crucial for the function of epithelial cells. Several models for the formation and maintenance of epithelial domains have been proposed, such as targeted vesicle trafficking, lateral sorting of proteins within the membrane or physical diffusion barriers at the membrane.

We investigate the formation of epithelial domains during cellularization in blastoderm embryos. Early Drosophila embryos switch from syncytial to cellular development during the mid-blastula transition. Concomitant with onset of zygotic gene expression, the plasma membrane ingresses between adjacent nuclei. Linked to furrow invagination is the establishment of membrane domains. During this stage, the epithelial compartments are set up *de novo* with a stereotypic time course, while the first epithelium is formed. Here, we investigate the dynamics and genetic control of how basal and lateral marker proteins (Slam versus Dlg) and subapical and lateral markers (Baz, Canoe versus Dlg, Scribble) are separated.

We have found that the actin nucleator and elongator Diaphanous (Dia) is required for formation of both boundaries. In *dia* mutant embryos, lateral proteins spread into the basal domain, as we reported previously and subapical determinants, such as Baz and Canoe are widely distributed throughout the lateral domain. By live imaging, we have found that the protein Canoe already segregates to a specific domain within the first few minutes of cellularization concomitantly to basal segregation of Slam, whereas the sub-apical determinant Baz accumulates only 20 min later. As both, Canoe and Baz, do not properly segregate in *dia* mutants, *dia* appears to function upstream in the genetic pathway.

We will present our ongoing experiments concerning the mechanism, how *dia* permits segregation of membrane domains and our analysis of components acting upstream of *canoe* that confer the positional information for the subapical domain. We identified a putative Rap1-GEF with subapical enrichment that is required for the restricted subapical Canoe localization.

80 C-terminal Src kinase (Csk) regulates the tricellular junctional protein Gliotactin independent of Src *G.D.N.Gayathri Samarasekera*, Vanessa Auld. Zoology, University of British Columbia, Vancouver, BC, Canada.

The permeability barriers created by the septate junctions (SJs) in invertebrate epithelia and tight junctions (TJs) in vertebrate epithelia are vital for the survival of animals. Likewise permeability barriers are formed by tricellular junctions formed at the corners of polarized epithelia where tight junctions or septate junctions converge. Gliotactin (Gli) is a transmembrane protein found at tricellular septate junctions in Drosophila and is essential for the maturation and maintenance of both bicellular and tricellular septate junctions. However, overexpression of Gliotactin leads to the spread of Gli away from the TCJ and disrupts epithelial architecture by signaling for overproliferation, delamination, migration and apoptosis of Gliotactin overexpressing cells. This leads to the suggestion that the level and the unique localization of Gliotactin needs to be tightly controlled. Gliotactin is downregulated through phosphorylation of two highly conserved tyrosine residues and subsequent endocytosis. Further, Gliotactin overexpression phenotypes depend on phosphorylation of septate junction associated protein Dlg. However, kinases involved in either case have not been identified yet. Therefore, a kinase RNAi screen was done in Gli overexpressed background using Drosophila columnar epithelia in wing imaginal disc. Suppressors and enhancers of Gli phenotypes were identified by screening 200 kinases. Among them, downregulation of C-terminal Src kinase (CSK) greatly enhanced Gli phenotypes suggesting that CSK might be a kinase responsible for endocytosis mediated downregulation of Gliotactin. Using Proximity ligation assay we showed that the degree of tyrosine phosphorylation associated with Gliotactin is increased when Csk is co-expressed with Gliotactin leading to suppression of the Gliotactin-induced overexpression phenotypes. More importantly, we found that Csk is required for the regulation of both overexpressed and endogenous Gliotactin. When Csk is downregulated in otherwise wild type background, Gliotactin spreads away from tricellular junctions. Although, Csk is well known as a negative regulator of Src kinases, we found that the effects of Csk on Gliotactin are independent of Src. Our data suggests that CSK controls wide range of proteins beyond Src and is necessary for the regulation of tricellular junctions.

81 Marf-mediated mitochondrial fusion is imperative for the development and functioning of the indirect flight muscles (IFMs) in *Drosophila* *Prasanna Katti*, Upendra Nongthomba. Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, Karnataka, India.

Mitochondrial function is dependent on the continual changes in mitochondrial morphology that occur in response to tissue requirements. In an energetically active tissue such as muscle, proper mitochondrial function is needed to sustain muscle activity and *vice versa,* cues from the muscle control mitochondrial health. The maintenance of mitochondrial quality and quantity in muscle involves the dynamic interplay between fusion and fission. However, the mechanisms by which mitochondrial shape and size are alternatively changed and maintained *in vivo* during myogenesis and, how the muscle-specific dynamic changes in mitochondrial morphology and numbers affect the tissue developmental processes have not been well studied. Here, we have used the *Drosophila* IFMs as a model system to study the significance of fusion and fission during muscle development and resultant impact on muscle function. During IFM development, the size and shape of mitochondria undergo characteristic change indicative of both fusion and fission. In *Drosophila*, mitochondrial fusion is regulated by Mitochondrial associated regulatory factor (Marf) and Optic atrophy 1-like (Opa1-like) proteins and fission by Dynamin related protein 1 (Drp1). We found that Marf expression during early IFM development is necessary for the fusion process to take place in later stages of development and for the mitochondria to attain the characteristic adult morphology. Marf is required during myofibrillogenesis for normal mitochondrial morphology and its loss resulted in dysfunctional mitochondria that underwent mitophagy. However, depletion of Marf during later stage of IFM development had no consequence on fusion or the adult mitochondrial morphology, suggesting that maintaining sustained expression of Marf during later stages of IFM development was not necessary. We also show that Marf is needed in IFMs throughout development not just for the regulation of mitochondrial morphology, but also for the functioning of the IFMs. Reduction in Marf during myogenesis caused decrease in myofibre length and sarcomeric width. These thinner myofibres with defective mitochondria were unable to support muscle function resulting in flightless adult flies. On the other hand, knock down of Drp1, the regulator of fission, did not affect the mitochondrial morphology, muscle function and myofibril ultrastructure of the developing IFMs. Therefore, the spatio-temporal regulation of the expression of Marf and mitochondrial fusion, but not the Drp1 and mitochondrial fission, appears to be critical for the development and functioning of the IFMs.

82 The relationship between autophagy, Rab-mediated endosomal trafficking, and T-tubule remodeling in muscles. *Tzu-Han Lin*[1], Naonobu Fujita[2], Amy Kiger[1]. 1) Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA, 92093 USA; 2) Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai, Miyagi 980-8578, Japan.

T-tubules are specialized plasma membrane invaginations in muscle cells critical for contraction, yet T-tubule

formation and remodeling are poorly understood. We identified a developmental myofiber remodeling program during Drosophila metamorphosis with distinct membrane trafficking requirements for T-tubule disassembly and reassembly. Through a timecourse analysis by live cell microscopy imaging in intact myofibers, we observed an upregulation in both endosomal and autophagy membrane flux coincident with the timing of T-tubule membrane disassembly. Muscle-targeted disruption of genes involved in autophagy initiation (Atg1, Atg18, Atg3) revealed an essential autophagy role for proper T-tubule membrane disassembly and progression in myofiber remodeling. Through RNAi screens, we identified Rab35 and Rab2 requirements in T-tubule remodeling. We pinpointed both Rab35 and Rab2 functions are needed specifically during autophagy-mediated T-tubule disassembly, with RNAi defects in both endosomal and autophagy membrane flux. Currently, we are testing the relationship between T-tubule derived membrane as a coordinated source for both endo-lysosomal and autophagosomal membranes during the remodeling process. Our further discovery of broader tissue roles for Rab35 and Rab2 in late stages in autophagy underscore the value of myofiber remodeling to understand muscle maintenance, as well as is a sensitized system for illuminating fundamental membrane trafficking mechanisms.

83 Social experience and hormone signaling modulate *fru*^{M} expression in the adult olfactory system. Pelin C Volkan[1], *Songhui Zhao*[1], Bryson Deanhardt[2]. 1) Duke University, Department of Biology, DURHAM, NC; 2) Duke University, Department of Neurobiology, DURHAM, NC.

Courtship behaviors in *Drosophila* are regulated by a single gene *fruitless (fru)*, where sex-specific alternative splicing *of fru* generates the protein product Fru^M only in males. Fru^M function is necessary and sufficient to drive male-specific courtship behaviors. *fru*^{M} is expressed in two thousand interconnected neurons in a sexually dimorphic manner in the *Drosophila* nervous system, acting as a molecular marker that labels neurons in the circuits that control sex-specific behaviors.

Olfaction is a key regulator of courtship behaviors, as flies detect volatile pheromones and other odors important for the initiation of courtship via olfactory receptor neurons (ORNs). In the olfactory system three ORN classes, expressing Or67d, Or47b, and Ir84a, are associated with courtship behaviors and express *fru*^{M}. We recently showed that expression of *fru*^{M} in Or47b and Ir84a ORNs requires Or47b and Ir84a signaling, respectively, through CaMKI and histone acetyl transferase p300. This is in contrast to Or67d ORNs, where *fru*^{M} expression is independent of Or67d function. This difference in *fru*^{M} regulation might reflect functional differences of Or47b and Ir84a ORNs, which modulate courtship behaviors based on olfactory experience indicating social and food related cues, from Or67d ORNs, which regulate innate aspects of courtship behaviors. Indeed, Or47b detects pheromones, particularly palmitoleic acid (PA), and is required for the increase in courtship and copulation advantage of older males. This change in courtship behavior is due to changes in Or47b ORN neurophysiology partly driven by juvenile hormone receptor *methoprene-tolerant* (*met*), an HLH-PAS transcription factor. We predicted that one mechanism by which Or47b and Met can modify male courtship behaviors is through reprogramming *fru*^{M} expression in OR47b ORNs using social olfactory experience and hormone signaling. Fru^M then can transcriptionally regulate the expression of genes, which can modify neurophysiology and courtship behaviors. In agreement with this, in addition to Or47b signaling, we found Met function to be required for *fru*^{M} expression in adult Or47b ORNs.
Furthermore, *fru*^{M} expression in OR47b ORNs increases with group housing from day 1 to day 5; and remains around day 1 levels when flies are socially isolated. Our data suggest that pheromone signaling of social environment through calcium activity and chromatin modulation, together with hormone signaling, reprogram *fru*^{M} expression in Or47b ORNs to drive behavioral adaptations.

84 The Role of Highly Conserved miRNAs in Tuning Synaptogenesis through Target Regulation in Specific Tissue Compartments *E. McNeill*[1], S. Alkins[2], A. Taylor[1], C. Warinner[1], H. Heggeness[1], L. Griffith[2], D. VanVactor[1]. 1) Cell Biology, Harvard Medical School, Boston, MA; 2) Biology, Brandeis University, Waltham, MA.

MicroRNAs (miRNAs) are well known players in facilitating the formation of neuronal connections. Several individual miRNAs have been demonstrated to function in synaptic development and maintenance. However, a comprehensive loss of function screen *in vivo* has not been possible without new technological advances. With a powerful combination of conditional inhibition (miRNA sponges) and miRNA nulls across a significant portion of the conserved miRNAs, we are able to assess broad miRNA involvement in synaptogenesis with spatial and temporal specificity for the first time. In this work, we examine 131 conditional miRNA sponges (miR-SP) targeting well-conserved miRNAs in *Drosophila* and validate hits from the ubiquitously expressed miR-SP screen at the neuromuscular junction (NMJ) using the complimentary miRNA null collection. Our unbiased screen analysis identified 10 novel miRNAs involved in synaptogenesis illustrating a rich regulatory network. Further analysis of tissue specific requirements of miRNAs reveals opposing growth phenotypes on the presynaptic and postsynaptic sides and corresponding tissue-specific regulatory targets in a human conserved miRNA. As a whole, this work not only illustrates the extensive regulatory complexity at the synapse by miRNAs, but also expands our understanding of how a miRNA can work simultaneously in separate tissue compartments to regulate synaptic form and function.

85 Sidekick is required to establish the circuitry for visual motion detection in *Drosophila* Sergio Astigarraga[1], Jessica Douthit[1], Dorota Tarnogorska[2], Matthew Creamer[3], Omer Mano[3], Damon Clark[3], Ian Meinertzhagen[2], *Jessica Treisman*[1]. 1) Cell Biology/Skirball Institute, New York University School of Medicine, New York, NY; 2) Psychology and Neuroscience, Dalhousie University, Halifax, NS, Canada; 3) Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Assembly of a functional neural circuit requires neurons to extend growth cones in defined directions and to recognize the appropriate synaptic partners through adhesion molecules on the cell surface. Vertebrate Sidekick proteins are immunoglobulin superfamily members that mediate the formation of specific synapses involved in visual motion detection. We have found that the single *Drosophila* Sidekick is a homophilic adhesion molecule that accumulates in specific synaptic layers of the visual motion detection circuit and is required for the normal behavioral response to motion stimuli. *sidekick* is required in photoreceptors, but not lamina neurons, both for the alignment of lamina neurons into columns and for the later sorting of photoreceptor axons into lamina cartridges based on their precise spatial orientation. Sidekick present at contacts between photoreceptor growth cones in the lamina may provide stabilizing adhesion that permits the growth cone fronts to extend in stereotyped directions. Sidekick is also localized to the dendrites of T4 and T5, the output cells of the ON and OFF motion detection circuits, and is expressed in some of their presynaptic partners. However, it is not essential for T4 and T5 to direct their dendrites to the appropriate layer or to receive synaptic contacts. Although the requirement for Sidekicks in establishing visual motion detection circuits is conserved, our results suggest that *Drosophila* Sidekick mediates adhesion that contributes to the organization of neuronal processing units, while its more diverse vertebrate homologues are used to select synaptic partners.

86 Frazzled promotes growth cone attachment at the source of a Netrin gradient in the *Drosophila* visual system *Orkun Akin*, S. Lawrence Zipursky. Department of Biological Chemistry, Howard Hughes Medical Institute, UCLA, Los Angeles, CA.

Axon guidance is proposed to act through a combination of long- and short-range attractive and repulsive cues. The ligand-receptor pair, Netrin (Net) and Frazzled (Fra) (DCC, Deleted in Colorectal Cancer, in vertebrates), is recognized as the prototypical effector of chemoattraction, with roles in both long- and short-range guidance. In the *Drosophila* visual system, R8 photoreceptor growth cones were shown to require Net-Fra to reach their target, the peak of a Net gradient. Using live imaging, we show, however, that R8 growth cones reach and recognize their target without Net, Fra, or Trim9, a conserved binding partner of Fra, but do not remain attached to it. Thus, despite the graded ligand distribution along the guidance path, Net-Fra is not used for chemoattraction. Based on findings in other systems, we propose that adhesion to substrate-bound Net underlies both long- and short-range Net-Fra-dependent guidance *in vivo,* thereby eroding the distinction between them.

87 Targeting without a target: How postsynaptic neurons guide photoreceptors in *Drosophila* visual map formation *Egemen Agi*[1,2], Charlotte Wit[2], Peter Robin Hiesinger[2]. 1) Neuroscience Graduate Program at UT Southwestern Medical Center, Dallas, TX 75390, USA; 2) Division of Neurobiology, Institute for Biology, and NeuroCure Cluster of Excellence, Freie Universität Berlin, 14195 Berlin, Germany.

The developmental outcome of neural circuit assembly is the correct matching of pre- and postsynaptic partners. This synaptic specification process can be facilitated by axonal and dendritic growth that pre-sorts correct synaptic partners into spatial vicinity. Presynaptic target identification typically requires the postsynaptic side as a target. Here, we report the surprising finding that around 4500 presynaptic photoreceptors (PRs) during *Drosophila* visual map formation do not require their main postsynaptic neurons, the lamina cells (L-cells), as a target.

During development, PR axons from a single unit eye arrive the brain as a bundle of six and subsequently re-sort with 750 bundles of PRs from all unit eyes according to the principle of neural superposition. Using intravital imaging in intact, developing pupae, we show that L-cells form a highly overlapping, dynamic, and patterned filopodial mesh at the time when PR neurons sort. Ablation of all L-cells in individual cartridges revealed no defects of PRs sorting into these cartridges, but targeting defects in surrounding cartridges. This finding suggests an early role for L-cells in guiding PR growth cones, but no late role as a target. To dissect these roles, we blocked L-cell filopodial dynamics, using dominant shibire^{ts} expression and intravital imaging, during different developmental time windows. Indeed, L-cell filopodial dynamics were not required either during PR growth cone extension or for them to stop in the correct target cartridge. In contrast, L-cell filopodial dynamics were required prior to PR growth cone extension for the formation of an L-cell grid, which not only arranges intra-bundle PR positions but also establishes regular spacing between PR bundles. These data suggest that lamina cells are important for PR targeting through early pattern generation and possibly the determination of PR extension angles. Our findings support a model in which synaptic specificity in the fly visual map is largely governed by pattern formation rules, rather than classical target recognition.

88 *slit* is required for proper lch5 chordotonal neuron morphology and migration in the Drosophila embryonic PNS *Madison L Gonsior*, Afshan Ismat. Department of Biology, University of St. Thomas, St. Paul,

MN.

Cells migrate along pathways to their target during embryogenesis, responding to different repulsive and attractive cues in their environment. The Slit-Robo signaling pathway acts to repel axons away from the midline of the central nervous system (CNS) via Robo, a transmembrane protein, and Slit, a secreted protein in the extracellular matrix (ECM). In the peripheral nervous system (PNS), the lateral chordotonal neurons (lch5) are a group of five neurons that repel away from the midline of the CNS while migrating laterally. The absence of *slit* showed a change in the lch5 migration pattern. The lch5 neurons migrated more dorsally and were not aligned properly. Moreover, the morphology of the lch5 neurons was also altered in the absence of *slit*. Specifically, the lch5 neurons did not display a teardrop shape, did not interact with each other properly, and their dendrites were pointing in various directions. These results suggest an important role for *slit* in sensory neuron migration and morphology. Further work needs to be done in order to elucidate the exact mechanism of lch5 chordotonal neuron migration.

89 Depolarization-dependent hyperacidifcation of dopamine synaptic vesicles is mediated by VGLUT Jenny Aguilar[1], Matthew Dunn[2], Susana Mingote[2], Caline Karam[2], Zachary Farino[3], Mark Sonders[2], Anna Grygoruk[4], Yuchao Zhang[2], Carolina Cela[5], Ben Choi[2], Jorge Flores[2], Brian McCabe[5], David Krantz[4], Jonathan Javitch[2], David Sulzer[2], Dalibor Sames[2], Stephen Rayport[2], *Zachary Freyberg*[3]. 1) Vanderbilt University, Nashville, TN; 2) Columbia University, New York, NY; 3) University of Pittsburgh, Pittsburgh, PA; 4) University of California Los Angeles, Los Angeles, CA; 5) EPFL, Lausanne, Switzerland.

The ability of presynaptic dopamine terminals to tune neurotransmitter release to meet the demands of neuronal stimulation is critical to neurotransmission. Although vesicle content was assumed to be static, *in vitro* data increasingly suggests that cell activity modulates vesicle content. Here we use novel genetic, pharmacological and imaging approaches in *Drosophila* to study *in vivo* the presynaptic machinery responsible for these vesicular processes. We show that cell depolarization increases synaptic vesicle dopamine content via vesicular hyperacidification prior to release. We also find that the vesicular glutamate transporter (VGLUT) is required for this stimulation-induced hyperacidification in *Drosophila* dopamine terminals. Furthermore, we demonstrate that both stimulation-induced dopamine vesicle hyperacidification and its dependence on VGLUT2 are conserved in mammalian brain. Together, these data suggest that a cascade of ion transport across vesicular transporters including VGLUT increases the vesicular pH gradient, thereby increasing DA vesicle content in response to neuronal depolarization.

90 Draper Expression in Cortex Glia Is Required for Dead Neural Cell Removal in the Developing *Drosophila* Optic Lobe *Ryosuke Nakano*[1,2], Masashi Iwamura[1], Akiko Obikawa[1,2], Atsushi Terauchi[1], Yu Togane[1], Yusuke Hara[1], Toshiyuki Fukuhara[1], Masatoshi Tomaru[2], Toshiyuki Takano-Shimizu[2], Hidenobu Tsujimura[1]. 1) Developmental Biology, Tokyo University of Agriculture and Technology, Tokyo, Japan; 2) Applied Biology, Kyoto Intsitute of Technology, Kyoto-shi, Kyoto, Japan.

Due to the large number of neuronal cells that die through programmed cell death in the developing *Drosophila* optic lobe, immediate clearance of dead cells is essential for the normal development and function of the optic lobe. Our previous studies have shown that *draper* (orthologue of CED-1/JEDI/MEGF-10) is expressed in the optic lobe and required for the engulfment of dead cells. Draper expression is required at late larval and early pupal stages and the clearance process is completed by the middle pupal stages. Signaling molecules downstream of Draper, Shark, Ced-6, Crk/Mbc/Dced-12 and Rac1, also play roles in the clearance process. Ligand candidates for Draper, Pretaporter and CaBP1, showed little involvement in the clearance process. Our studies reported here identify phosphatidylserine as a potential ligand for Draper.

Here we analyzed the expression pattern of Draper in glia subtypes required for the clearance process following apoptosis. Draper was strongly expressed in neuro-epithelium, neuroblasts and lamina neural cells at the puparium formation. Draper expression in the cortex glia was weak at early pupal stages, but expression increased after 24h APF, and expression in the neuropil also increased after 72h APF. Next, we identified GAL4 drivers specific to glia subtypes, and analyzed the role of each subtype in the clearance process. This analysis indicated that Draper expressed in the cortex glia is necessary and sufficient for the clearance of dead neural cells during optic lobe development. Other glia subtypes failed to demonstrate a role in engulfment of dead cells. In summary, we have identified a unique and novel function for cortex glia cells. As such, our results provide new insights into glia cell function in the developing *Drosophila* nervous system.

91 Elucidating Recent Gene Flow Across Drosophila Species Using a Novel Machine Learning Approach *Daniel Schrider*[1,2], Andrew Kern[1,2]. 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ.

Gene flow between recently diverged species pairs has a profound influence on the speciation process. Moreover adaptive introgression, in which beneficial alleles cross species boundaries, appears to be commonplace in nature. Thus it is crucial to develop the statistical machinery required to uncover which genomic regions have

recently acquired haplotypes via introgression from a sister species. We developed a novel machine learning framework capable of uncovering genomic introgression with far greater power than competing methods. This method works by combining information from a number of population genetic summary statistics that capture patterns of variation across two populations, along with several additional novel statistics that we have devised for this work and show to be highly sensitive to introgression. In particular, this approach is able to detect with unparalleled accuracy which loci that have recently been exchanged among related species, and in many situations can correctly infer which population was the donor and which was the recipient. We applied this method to whole-genome sequence data in order to identify gene flow across the *D. melanogaster* subgroup, revealing numerous introgression events between members of this clade. We discuss putatively introgressed loci and their evolutionary implications with respect to the maintenance of species boundaries, and also highlight several candidate adaptive introgression events. More generally, the success of our approach demonstrates the remarkable potential of machine learning for population genetic inference.

92 Reinforcement of conspecific sperm precedence weakens sexual selection in sympatric populations of *Drosophila* *Dean M. Castillo*[1,2], Leonie C. Moyle[2]. 1) Cornell University, Ithaca, NY; 2) Indiana University, Bloomington, IN.

While sexual selection can drive the evolution of reproductive isolation, selection for increased reproductive isolation could also impact sexual selection when these processes share a genetic basis. Direct selection for isolation is most likely to occur in the context of 'reinforcement', where selection acts to increase pre-zygotic barriers to avoid costly heterospecific matings. Post-mating traits, such as conspecific sperm precedence (CSP), are among the barriers that can potentially respond to reinforcing selection, and as a consequence of the shared genetic basis between CSP and intrapopulation sperm competition (ISC), selection for increased CSP in sympatric populations could alter ISC and sexual selection. We test this expectation with the sister species *Drosophila pseudoobscura* and *D. persimilis*, using two sympatric and two allopatric populations of *D. pseudoobscura*. We used a factorial sperm competition experiment to estimate differences in the mean and variance for CSP and ISC between sympatric and allopatric populations, and the genotype contributions of each sex to CSP and ISC. Using multiple tester males across this factorial design also allowed us to estimate the opportunity of sexual selection in each population. In parallel, we sequenced whole fly transcriptomes for both males and females of all genotypes to examine evidence that divergence at the transcript and sequence level shaped these phenotypic patterns. Consistent with a pattern of reinforcement, the sympatric populations had higher mean CSP and lower phenotypic variance compared to allopatric populations. Sympatric populations also showed decreased average offensive sperm competitive ability (ISC), suggesting that reinforcement of CSP constrained the opportunity for sexual selection specifically within sympatric populations. Unique candidate genes, including known accessory gland proteins with roles in ISC, were differentially expressed in each sympatric population. These data demonstrate that strong reinforcing selection for reproductive isolation can have consequences for sexual selection and sexual interactions, in these important postmating sperm competition traits, and that the genes that respond to this selection may differ between sympatric populations.

93 Unorthodox transmission modes of endosymbionts in hybrids and the symbiotic origin of speciation *Wolfgang Miller*[1], Lisa Klasson[2], Daniela Schneider[1], Lee Ehrman[3]. 1) Lab Genome Dynamics, Center of Cell and Developmental Biology, Medical Univ Vienna, Vienna, Austria; 2) Department of Cell and Molecular Department of Molecular Evolution, Molecular Evolution, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden; 3) Natural Sciences, State University of New York, Purchase College, Purchase, New York, USA.

Although not a new idea, recent studies suggest that differences in the composition of symbiotic microbes between hosts can lead to reproductive isolation, and as a consequence also to speciation. Despite the fact that microbes are universally present in eukaryotes, they are rarely considered as a driving force of speciation, and chances are their contribution to speciation is overlooked. Symbiotic bacteria of the genus *Wolbachia* are known to affect their hosts' reproduction in adaptive manners to improve the propagation of the maternally transmitted endosymbiont throughout populations. These reproductive alterations that can result in postmating isolation *via* cytoplasmic incompatibilities, have recently been shown to foster also premating isolation in some host-symbiont associations such as the *Drosophila paulistorum* species complex, giving even more reason to assume that *Wolbachia* can play a significant role in host speciation.

Here we will present most recent data on the involvement of *Wolbachia* in host speciation in Neotropical Drosophila species that are under incipient speciation in nature, carrying closely related but incompatible *Wolbachia* strains. We will also show that naturally incompatible and sterile interspecies hybrids can be rescued by means of mild paternal *Wolbachia*-knockdown before forced mating, giving rise to fertile progeny and thereby stable hybrid lines via sib mating. Even more surprisingly, such rescued hybrid lines show complete sexual isolation to their parental lines

plus unambiguous signatures of paternal inheritance of both their cytoplasmic endosymbionts, *i.e.*, of mitochondria and *Wolbachia*.

94 Global Patterns of Local Ancestry in Drosophila melanogaster *Russ Corbett-Detig*. Biomolecular Engineering UC Santa Cruz, Santa Cruz, CA.

Admixture---the mixing of genetically divergent populations---is increasingly recognized as a central process in evolution. In natural populations of *Drosophila melanogaster*, ancestral African and Cosmopolitan populations have recurrently encountered each other and hybridized, making this an ideal system to dissect the consequences of admixture. Here, we develop methods to analyze patterns of heterozygosity, identity by descent, and admixture in approximately 1,000 genomes in the *Drosophila* Genome Nexus database. Our results indicate that admixture events in sub-Saharan Africa are recent, on the order of 1,000 generations or fewer. Furthermore, we find compelling evidence that natural selection has shaped patterns of local ancestry in African populations including a significant contribution of adaptive introgression of non-African alleles. Among other biological processes, histone modifying proteins are enriched within ancestry frequency outlier regions, suggesting this is an important component of selection on local ancestry in admixed populations of *D. melanogaster*.

95 Non-neutral species-specific gene death causes hybrid male sterility in *Drosophila* *Emily L. Landeen*[1], Christina A. Muirhead[2,3], Lori Wright[2], Thomas Rzatkiewicz[2], Daven C. Presgraves[2]. 1) University of California Berkeley, Berkeley, CA; 2) University of Rochester, Rochester, NY; 3) The Ronin Institute, Montclair, NJ.

A central goal in evolutionary biology is to determine the molecular evolutionary basis of speciation. Multiple lines of evidence across taxa show that the X chromosome plays a special role in the evolution of hybrid male sterility, although the molecular genetic basis of this so-called “large X effect” remains unclear. We performed comparative genetic mapping of hybrid male sterility for an X-linked region between *D. mauritiana* and *D. simulans*, and between *D. mauritiana* and *D. sechellia.* We identify and transgenically validate a gene, which we call *Sirius*, that causes complete hybrid male sterility when introgressed from *D mauritiana* into *D. simulans. Sirius* is a testis-specific gene that shows a remarkable structural difference between species. In *D. simulans* and *D. sechellia*, *Sirius* is a 2.0-kb intronless, protein-coding gene. However, in *D. mauritiana*, 480 to 1833 bp (23-89%) of the *Sirius* transcript, including the start codon, has been eliminated by at least five large deletion events that segregate in the population. PCR and BLAST of deep-coverage PacBio genome sequence confirms *Sirius* has not been duplicated elsewhere in the genome. Population genomic analysis of comparable deletion events shows that the conspicuous concentration of large degenerative mutations overlapping the *Sirius* start codon is an extreme outlier, suggesting natural selection acted in the elimination of *Sirius* protein-coding capacity in the *D. mauritiana* lineage. Our results suggest that the turnover of X-linked male fertility-essential genes may play an important role in the evolution of the large X effect.

96 Genetic epistasis within regulatory regions acts to buffers the effect of segregating mutations during embryonic development *Mattia Forneris*, Enrico Cannavo, Dermot Harnett, Ignacio Schor, Nils Koelling, Ewan Birney, Oliver Stegle, Eileen EM Furlong. European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany.

Embryonic development is driven by precise patterns of gene expression, which are tightly regulated despite extensive genetic variation among individuals. Expression Quantitative Trait Loci (eQTL) studies indicate that sequence variation altering gene expression is relatively frequent in cell-culture models and differentiated tissues. How such variation impacts, and is buffered within, developmental programs remains unclear. To study the extent and types of genetic variation impacting embryonic gene expression we designed two complementary QTL studies, examining the effect of genetic variation on multiple molecular phenotypes, including transcriptional (expression levels, transcriptional start site (TSS) usage) and post-transcriptional (3′ RNA processing) regulation. We measured variation across 80 inbred *Drosophila* wild isolates at three stages of embryogenesis, identifying thousands of developmental-stage-specific and shared quantitative trait loci (QTL). Given the small blocks of linkage disequilibrium in *Drosophila* we obtain near base-pair resolution, resolving causal mutations in development enhancers, validated Transcription Factors Binding Sites (TFBS), and motifs involved RNA processing and 3′ UTR length change. Using single-cell measurements and extensive engineering, where we place the minor allele into the major genotype, we uncover two interesting properties of regulatory elements during embryonic development: First, extensive epistasis within enhancer elements, which acts to partially buffer the impact of regulatory mutations. Second, QTL that impact the distribution of transcriptional start sites (TSS) within promoters (a property known as promoter shape) often increase expression noise. Similar to developmental enhancers, we find heteroallelic interactions with other promoter variants that partially buffer each other’s effects. Taken together our results indicate that large effect variants are more likely to segregate when balanced against other variants with opposite effects. These results highlight how developmental context conditions the impact of genetic variation, and uncovers multiple mechanisms that regulate and buffer expression changes during embryonic development

97 Contingency and convergence in the evolution of regulatory sequence: Dosage compensation in Drosophila *Christopher Ellison*[1], Doris Bachtrog[2]. 1) Department of Genetics, Rutgers University, New Brunswick, NJ; 2) Department of Integrative Biology, University of California at Berkeley, Berkeley, CA.

Dosage compensation in Drosophila is acheived via the recruitment of the chromatin modifying MSL complex to high affinity locations along the male X chromosome termed chromatin entry sites (CES). The MSL complex recognizes a specific DNA sequence motif within these CES known as the MSL recognition element or MRE motif. We previously found that CES evolved on the *D. miranda* neo-X chromosome when a transposable element dispersed an MRE-like sequence motif across the chromosome, suggesting that transposable elements may play an important role in the formation of CES in Drosophila and the evolution of new regulatory sequence in general. However, a series of recent studies by other groups has provided evidence that MRE motifs more often arise from the polypyrimidine tracts of introns or via the expansion of GA dinucleotides. To determine the relative importance of these various mechanisms of MRE evolution, we have used Chromatin isolation by RNA Purification (ChIRP) to characterize MSL binding in three closely related species within the robusta group of Drosophila, each of which has an independently formed neo-X chromosome. We observe strong conservation of CES location on the ancient X chromosomes of these species and a striking convergence of CES location on their neo-X chromosomes. We find that MRE motifs evolve via a variety of mechanisms, whose relative importance appears to depend on historical features of the species in question, such as repetitive DNA content, suggesting that historical contingency may play an important role in the evolution of regulatory sequence.

98 Determination of EGFR Signaling Output by Opposing Gradients of BMP and JAK/STAT Activity. *Laura A Nilson*[1], Mariana Fregoso Lomas[1], Scott De Vito[1], Jean-Francois Boisclair Lachance[2], Josee Houde[1]. 1) Department of Biology, McGill University, Montreal, QC, Canada; 2) Ben May Department for Cancer Research, The University of Chicago, Chicago, IL.

A relatively small number of signaling pathways drive a wide range of developmental decisions, but how this versatility in signaling outcome is generated is not clear. In the Drosophila follicular epithelium, localized epidermal growth factor receptor (EGFR) activation induces distinct cell fates depending on its location. Posterior follicle cells respond to EGFR activity by expressing the T-box transcription factors Midline and H15, while anterior cells respond by expressing the homeodomain transcription factor Mirror. We show that the choice between these alternative outputs of EGFR signaling is regulated by antiparallel gradients of JAK/STAT and BMP pathway activity and that mutual repression between Midline/H15 and Mirror generates a bistable switch that toggles between alternative EGFR signaling outcomes. JAK/STAT and BMP pathway input is integrated through their joint and opposing regulation of both sides of this switch. By converting this positional information into a binary decision between EGFR signaling outcomes, this regulatory network ultimately allows the same ligand-receptor pair to establish both the anterior-posterior (AP) and dorsal-ventral (DV) axes of the tissue.

99 Proximodistal patterning of the fly leg relies on tight spatiotemporal regulation of two key EGFR inputs via leg disc-specific, non-redundant enhancers *Susan Tozier*[1], Roumen Voutev[2], Richard S. Mann[2]. 1) Biological Sciences, Columbia University, New York, NY; 2) Biochemistry & Molecular Biophysics, Columbia University Medical Center, New York, NY.

EGFR signaling is responsible for patterning the proximal-to-distal (P-D) axis of the fly leg. Proper patterning relies on tightly controlled expression of at least two key EGFR inputs, Vein and Rhomboid, which encode an EGFR ligand and activating protease, respectively. We describe the identification and the direct inputs into the cis-regulatory modules (CRMs) that mediate their leg-specific expression. Further, we used CRISPR/Cas9 to target and replace the endogenous CRMs with a platform for carrying out Recombination Mediated Cassette Exchange (RMCE), which allows these CRMs to be deleted or replaced by any sequence of interest and then observe the consequences for gene expression and patterning. When leg-specific CRMs from vein (*vein(leg)*) or rhomboid (*rho(leg)*) are deleted, their expression in leg imaginal discs is no longer observed, indicating that these enhancers are non-redundant. Further, *rho(leg)*; *vn(leg)* double enhancer deletions abolish distal leg fates suggesting that, although six additional Rho-family proteases are encoded in the *Drosophila* genome, Rhomboid and Vein are essential for regulating EGFR in leg P-D patterning. Since Rho proteases can activate any TGF-α-like ligand, we assessed discs mutant for subsets of these ligands, and find that animals with leg discs that are mutant for both *vein* and *spitz* also have compromised distal leg patterning. Finally, although leg P-D patterning relies on a central gradient of EGFR activity in the disc, it is not clear how such a gradient might be established given the known inputs. Having observed that expression of *vein* begins earlier and is more centrally constrained than that of *rhomboid*, we employed our CRM RMCE system to swap the sequences of *vein(leg)* and *rho(leg)* CRMs and assessed whether perturbing this difference in timing and breadth of expression affects EGFR target gene expression patterns or adult leg morphology. Together, these experiments provide insight into how EGFR signaling is regulated in time and space to specify the pattern of the leg.

100 Dynamic patterning by the *Drosophila* pair-rule network reconciles long-germ and short-germ segmentation *Erik Clark*, Michael Akam. Department of Zoology, University of Cambridge, Cambridge, United Kingdom.

During the third hour of development, the anterior-posterior axis of the *Drosophila* embryo is patterned down to cellular level resolution. Using spatial information from graded domains of "gap" gene expression, transcription factors known as the "pair-rule" genes are expressed in periodic patterns of seven stripes. The pair-rule genes then work in combination to specify precisely-phased 14 stripe patterns of "segment polarity" genes. These output patterns form the template for the segmental organisation of the insect body.

Although *Drosophila* segmentation is well-studied, important recent discoveries have been hard to fit into the textbook framework of *Drosophila* blastoderm patterning. First, quantitative studies have revealed that the early pair-rule stripes are not static, but instead shift anteriorly over time, driven by feedback interactions within the gap gene system. Second, comparative studies have shown that the *Drosophila* pair-rule genes must ancestrally have been involved in a clock-and-wavefront type segmentation mechanism, as their orthologs exhibit oscillatory expression in the posteriors of short-germ arthropod embryos.

We propose a revised view of *Drosophila* segment patterning that makes sense of these observations. Using a combination of genetic experiments and computational modelling, we have determined the topology of the pair-rule network, and simulated its dynamical behaviour. Our model reveals the functional significance of gap gene expression shifts for downstream patterning: only when key pair-rule inputs take the form of posterior-to-anterior kinematic waves can our simulations recapitulate observed gene expression patterns from either wild-type or mutant embryos. This is because spatial patterning of the *Drosophila* blastoderm appears to be a process with a fundamentally temporal basis, with required stripe phasings emerging from the dynamics of regulatory feedback within the pair-rule gene system itself.

Suggestively, we find that our model of the *Drosophila* pair-rule network can perform either simultaneous or sequential segmentation, depending only on initial conditions. We therefore propose that the patterning of the *Drosophila* blastoderm involves components of an ancestral segmentation clock, and hypothesise that the evolutionary transition from short-germ to long-germ segmentation has involved providing a largely conserved segmentation network with altered spatiotemporal inputs.

101 The LIM protein Smallish associates with Bazooka/Par-3 and Src at adherens junctions to control epithelial morphogenesis Hamze Beati[2,3], Irina Peek[1], Paulina Hordowska[2], Mona Honemann-Capito[2], Jade Glashauser[4], Fabian Renschler[5], Andreas Ramrath[7], Stefan Luschnig[4,6], Silke Wiesner[5], *Andreas Wodarz*[1,2,7]. 1) Molekulare Zellbiologie, Institut I für Anatomie, Universität zu Köln, Kerpener Str.62, 50937 Köln, Germany; 2) Stammzellbiologie, Institut für Anatomie und Zellbiologie, Georg-August Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany; 3) 8Entwicklungsgenetik, Institut für Biologie, Universität Kassel, Heinrich-Plett-Str. 40, 34132 Kassel, Germany; 4) Institute of Molecular Life Sciences (IMLS), University of Zurich, 8057 Zurich, Switzerland; 5) Max Planck Institute for Developmental Biology, Spemannstr. 35, 72076 Tübingen, Germany; 6) Institut für Neuro und Verhaltensbiologie, Westfälische Wilhelms-Universität Münster, Badestr. 9, 48149 Münster, Germany; 7) Institut für Genetik, Heinrich Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany.

In epithelia, cells adhere to each other in a dynamic fashion, allowing the cells to change their shape and to move along each other during morphogenesis. The regulation of adhesion occurs at the belt-shaped adherens junction (AJ), the zonula adherens (ZA). Formation of the ZA depends on components of the Par-aPKC complex of polarity regulators. We have identified the LIM protein Smallish (Smash), the ortholog of vertebrate LMO7, as an epithelium-specific binding partner of Bazooka/Par-3 (Baz), a core component of the Par-aPKC complex. Smash also binds to the tyrosine kinase Src42A, a known regulator of AJ dynamics. Animals lacking Smash show severe defects during embryonic morphogenesis characterized by the uncoordinated formation of epithelial invaginations and tubular organs. Overexpression of Smash causes apical constriction of epithelial cells. We propose that Smash is a key regulator of morphogenesis coordinating planar cell polarity, cadherin-based cell-cell adhesion and actomyosin contractility at the ZA.

102 Physical aspects of Drosophila gastrulation *Konstantin Doubrovinski*[1], Michael Swan[2], Eric Wieschaus[2], Reza Farhadifar[3], Daniel Needleman[3]. 1) Green Center for Systems Biology, UT Southwestern, Dallas, TX; 2) Department of Molecular Biology, Princeton University, Princeton NJ; 3) School of Engineering and Applied Sciences, Harvard University, Cambridge, MA.

Gastrulation is a process whereby a single layer of epithelial cells gives rise to a multilayered structure. In *Drosophila*, gastrulation starts with apical constriction of mesodermal cells followed by invagination of the mesodermal primordium into the interior of the embryo. Understanding the mechanism of *Drosophila* gastrulation requires answering two major questions. 1) Why does the mesoderm constrict along a particular axis? 2) Why does

the mesoderm invaginate?

To answer the first question, we measured mechanical properties of the mesoderm using an assay that involves injecting embryos with ferrofluid droplets. We then used magnetic force to pull on the ferrofluid droplets and measured the resulting deformation of surrounding tissue. Our measurements showed that the mesoderm is highly elastic. Based on our data, we propose a physical model where embryonic surface is represented by a flat elastic shell with a rectangular contractile domain. Our model explains the preferred axis of constriction based entirely on our measured data. Notably, our model explains the anisotropic constriction of the mesoderm without assuming any anisotropy in tension or material properties of cells. Furthermore, the tension within the mesoderm in this model is assumed to be uniform in space.

To address the question of why the mesoderm invaginates, we propose and experimentally test a physical model. A key feature of our model is that lateral membranes constrict actively. In our model, lateral membrane constriction is required for mesoderm invagination. In support of this, we present data where we ablate lateral membranes with micrometer precision and show that invagination stops if membranes are severed.

103 Transcriptional regulation of ribosomal protein genes is associated with organogenesis of secretory epithelium *R. Loganathan*[1], D. Johnson[1], M. Slattery[2], D. Andrew[1]. 1) Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Biomedical Sciences, University of Minnesota Medical School, Duluth, MN.

Organogenesis of secretory epithelia is critically dependent on the coordinate regulation of growth and form as the size and shape of secretory tissues are critical determinants of their functional complexity. Transcription factors that regulate the spatiotemporal patterns of gene expression during development affect multiple aspects of organogenesis including organ size and shape. In this work, we describe the distinct role of Ribbon (Rib), a BTB/POZ nuclear factor, during organogenesis of the *Drosophila* salivary gland (SG) — an unbranched tubular epithelium specialized for protein secretion. Our investigations of the embryonic SG demonstrate that Rib regulates cell growth during organogenesis. In *rib* null embryos with tube elongation defects, we observed significant changes in cell size and shape compared to the wild-type embryos. Notably, these changes in the *rib* mutants occurred without effects on cell specification, proliferation, apoptosis, apicobasal polarity and junctional integrity. How does Rib regulate cell growth in the secretory epithelium? To identify the transcriptional targets of Rib during SG organogenesis, we performed ChIP-Seq analysis in embryos driving *rib* expression specifically in the SG. Gene ontology characterization of the 494 genes bound by Rib in the embryonic SG assigned functional categories primarily spanning cell growth and morphogenesis. In particular, our data suggests that cell growth is achieved through the regulation of ribosomal protein genes by Rib. It is possible that Rib interacts with several known ribosomal protein gene regulatory motifs including the terminal oligopyrimidine sequence which has also been implicated in mammalian ribosomal protein gene regulation. Preliminary evidence suggests that the tissue-specific transcriptional regulation of translational machinery by Rib to achieve cell growth complements the SG-specific transcriptional program, mediated by CrebA, to enhance protein secretion. Collectively, these results suggest a mechanism for differentiating secretory cells to selectively regulate the translational machinery that prepares for the functional demand to produce and secrete very high levels of protein, concomitant with organ maturation.

104 Patterned Toll receptor expression organizes epithelial cell intercalation *Adam Pare*, Athea Vichas, Christopher Fincher, Zachary Mirman, Avantika Mainieri, Dene Farrell, Jennifer Zallen. Developmental Biology, Sloan Kettering Institute, NEW YORK, NY.

Tissue elongation through convergent extension is a common mechanism throughout animal development, and it requires the coordinated polarization and movement of large groups of cells. Elongation of the head-to-tail body axis in *Drosophila* is a striking example of convergent extension, and it involves the recruitment of contractile and adhesive proteins to distinct cell-cell interfaces, allowing cells to intercalate between one another in a globally organized manner. However, the mechanisms through which individual cells sense their spatial orientation with respect to the global body axes have long been mysterious. We recently showed that three members of the Toll receptor family, Toll-2, Toll-6, and Toll-8, are expressed in the early *Drosophila* embryo in repeating, partially overlapping stripes. We demonstrated that epithelial cells use this spatial code of cell surface receptors as a compass to determine directionality within the tissue. We propose a model in which heterophilic interactions between the extracellular domains of different Toll receptors on neighboring cells recruit contractile proteins and destabilize adhesive interactions at specific cell-cell interfaces, directing cell rearrangements to occur in a globally oriented manner. Toll family receptors have been well studied in the context of innate immunity, where they serve as crucial receptors for the detection of pathogen-derived molecules and endogenous damage-associated proteins. Our findings suggest a novel role for this protein family in mediating cell-cell interactions during normal development. Therefore, it appears that Toll receptors are used in a variety of cellular contexts, not only to distinguish self from non-self by the immune system, but also to distinguish cells from their neighbors. These studies point to a relatively

unexplored and potentially conserved link between Toll receptor signaling and activation of the actomyosin network to induce changes in cell shape and behavior. We are now using genetic, cell biological, and biochemical approaches to identify the signaling mechanisms by which Toll family receptors regulate cell polarity and tissue structure.

105 Transcription of the Y chromosome fertility factors – the role of intron gigantism and a potentially novel RNP granule *Jaclyn Fingerhut*[1,2], Yukiko Yamashita[1,2,3,4]. 1) Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI; 2) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 3) Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; 4) Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI.

The *Drosophila* Y chromosome encodes only a handful of genes, most of which are solely transcribed in spermatocytes and are essential for male fertility. Interestingly, most of these genes are embedded in heterochromatin and span several megabases. For example, one of these genes, *kl-3*, is 4.3Mb. However, its coding sequence is only 14kb (99.7% intronic). The introns of these Y chromosome genes are highly repetitive, containing megabases of satellite DNA repeats (e.g. $(AATAT)_n$). While intron gigantism is found on the Y chromosome of many *Drosophila* species, there is little sequence conservation within the introns and the effected intron (and gene) varies, raising the possibility that intron length is critical for the regulation of gene expression. However, little is known about how these unusually structured genes are regulated and whether intron size contributes to this regulation.

By using multi-color *in situ* hybridization to resolve the transcript originating from the first exon, the satellite DNA of the introns and a late exon, we found that transcription of *kl-3* is dynamically regulated during spermatocyte development, which spans 80-90 hours. Transcript from the first exon appears in early stage spermatocytes while transcript from late exons does not appear until the spermatocytes are almost ready to begin the meiotic divisions. This result raises the possibility that the intron length may be utilized to regulate the duration of transcription, possibly to delay the production of protein or to regulate spermatocyte development. Transcription of *kl-3* is dependent on spermatocyte-specific transcription factors.

Intriguingly, *kl-3* mRNA forms a cytoplasmic granule in late spermatocytes that segregates during the meiotic divisions and persists into spermiogenesis. This granule does not colocalize with known germ cell RNP granule components or with various organelles. Additionally, while it is known that spermatocytes transcribe almost all genes needed for meiosis and spermiogenesis, these transcripts appear diffuse in the cytoplasm, not as RNP granules. The only other component of this granule that we have identified thus far is *kl-2* mRNA (another Y chromosome gene). As *kl-3* and *kl-2* both code for axonemal dynein proteins, we hypothesize that this granule serves to store the axonemal mRNAs required for spermiogenesis for use during spermiogenesis.

106 A lipid metabolism checkpoint regulates self-renewal and differentiation of germline stem cells *Rafael S. Demarco*[1], Cecilia D'Alterio[1], Leanne Jones[1,2]. 1) Molecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA; 2) Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of California, Los Angeles.

Different metabolic states have been associated with the capacity of cells to reprogram, self-renew or differentiate. Most stem cells are thought to rely on glycolysis, with punctate primitive mitochondria not actively engaging in oxidative phosphorylation (OXPHOS). Upon differentiation, cells will reshape their mitochondrial network resulting in changes in metabolism and the activation of OXPHOS. However, other metabolic strategies can be utilized to regulate stem cell maintenance, activation, and the onset of differentiation. Here we show that *Drosophila* male germline stem cells (GSCs) rely on lipid metabolism for maintenance within the testis niche. An RNAi-based screen targeting regulators of mitochondrial dynamics (biogenesis, morphology, movement and turnover) revealed that mitochondrial fusion is required for GSC maintenance, and clonal analysis confirmed that GSCs mutant for Mitofusin (Mfn/Marf) are lost due to the premature onset of differentiation. Blocking mitochondrial fusion impaired mitochondrial activity and resulted in the accumulation of lipid droplets (LDs), which triggered arrest at the G1/S phase of the cell cycle. Either genetic or pharmacological enhancement of lipid utilization by the mitochondria rescued the loss of GSCs caused by the lack of mitochondrial fusion. Inhibiting fatty acid (FA) metabolism in GSCs directly, by RNAi-mediated depletion of the lipase brummer (bmm)/AtgL, the mitochondrial carnitine transporter congested-like trachea (colt), or the carnitine acyltransferase whithered (whd)/CPT1, also resulted in GSC loss and cell cycle arrest suggesting that a lipid metabolism checkpoint exists to ensure sufficient energy for tissue homeostasis. Our results demonstrate the importance of FA metabolism for GSC maintenance in *Drosophila*, which may be a conserved metabolic strategy utilized across organisms.

107 Zinc-Finger Transcription Factor Hindsight Regulates Ovulation Competency of *Drosophila* Follicles *Lylah Deady*[1], Wei Li[1], Jianjun Sun[1,2]. 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs, CT.

Ovulation is indispensable for successful reproduction and is conserved from insects to humans. For example, our recent work has demonstrated that *Drosophila* ovulation involves a breakdown of posterior follicle cells of the stage-

14 egg chambers, the rupture of the encapsulated oocyte into the oviduct, and the formation of a corpus luteum by the residual follicle cells, all reminiscent of mammalian ovulation. This follicle rupture is mediated by a conserved matrix metalloproteinase (Mmp2), which is specifically expressed in posterior follicle cells of stage-14 egg chambers. The signal to induce Mmp2 activation and follicle rupture is neuronal octopamine, which acts through the octopamine receptor in mushroom bodies (Oamb) on stage-14 follicle cells. However, it is still unknown how follicles become competent for ovulation. Here, we identified Hindsight (Hnt) as the first transcription factor regulating follicle's competency for ovulation. Hnt was not expressed in stage-13 follicle cells except those giving rise to dorsal appendage but upregulated in stage-14 follicle cells. Knocking down Hnt in stage-14 follicle cells significantly impairs ovulation. In addition, we found that upregulation of Hnt in stage 14 is essential for Mmp2 and Oamb expression. Furthermore, Hnt's role in regulating ovulation and Mmp2 and Oamb expression can be replaced by its human homolog Ras responsive element binding protein 1 (RREB-1), demonstrating the functional conservation between these two proteins. Our data suggest that Hnt primes the follicle to be competent to receive ovulation stimulus by regulating Oamb and Mmp2 expression, and its role may be conserved in mammalian ovulation.

108 Sex-specific specification of the follicle stem cells in the developing *Drosophila* ovary *Abigail Fuchsman*, Mark Van Doren. Biology Department, Johns Hopkins University, Baltimore, MD.

Sexual dimorphism is crucial for the propagation of a sexually reproducing species, thus understanding how sex-specific cells are specified is essential for our understanding of how an oocyte or sperm are produced. Our lab is interested in how the sex determination pathway controls sexual dimorphism in the gonad, including how the conserved transcription factor Doublesex (DSX) regulates sex-specific development of the somatic gonad. Follicle cells are female-specific cells that surround and nurture the developing oocyte and are conserved from flies to mammals. The germarium of the *Drosophila* ovary contains two follicle stem cells (FSCs) that give rise to the follicle cells, but how the FSCs are specified remains unknown. It is believed that the major somatic components of the germline niche, terminal filament cells in females and hub cells in males, come from the same precursor cells in the embryonic gonad. Additionally, the somatic cells that initially surround differentiating germ cells, the escort cells in females and cyst cells in males, express similar molecular markers and may have a common origin. However, the origins of the FSCs remain unknown, and we do not know the male equivalent to FCSs, if any exists. The best current marker for FSCs is the transcription factor Castor, which labels FSCs in addition to pre-follicle cells, and stalk cells. We have conducted a time-course immunostaining of pupal ovaries examining Castor expression as a readout for FSC specification. Castor is not observed at 2 hrs through 7 hrs after pupal formation APF. The earliest Castor expression can be seen at 9 hrs APF in cells intermingled with the germ cells in the middle of the developing ovarioles as well as in cells posterior to the germ cells. At 24 hrs APF, Castor expression is seen primarily in the basal stalk cells posterior to the germline. We are currently using lineage analysis to study the origins of the FSCs and if they are related to basal stalk cells. Once a follicle cell lineage is identified we can try to determine whether there is an equivalent cell type during male gonad development. Interestingly, we also find that many aspects of follicle cell development still occur specifically in XX animals even in the absence of *dsx* function. Thus, we are investigating whether there are *dsx*-independent mechanisms controlling sex-specific follicle cell development in the soma, or whether signaling from XX germ cells influences follicle cell development.

109 *Rap1* and Hippo pathway collaborate to polarize directional protrusions in *Drosophila* border cell migration *Yu-Chiuan Chang*[1], Yi-Chi Hsieh[2], Tzu-Han Huang[2], Zih-Min Liao[2], Yi-Shan Huang[2], Denise Montell[3], Anna C-C Jang[2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Institute of Biotechnology, National Cheng Kung University, Tainan, Taiwan; 3) Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA, USA.

Collective cell movement is a featured phenomenon in morphogenesis, wound healing and cancer invasion and metastasis. In most cases, cellular protrusions driven by actin polymerization orient and advance the coherent cells in response to external cues. Such directionality is achieved by coordinated polarity among the migrating cohort, failure of which causes migration delay or even group scattering. It remains unclear how the migrating cells polarize to adhere to the cluster center and to interact with the surroundings simultaneously and coordinately. Here we use *Drosophila* border cells as a model to study how group polarity is organized and regulates cell motility *in vivo*. With genetic analyses, live imaging, and biochemical studies, we unravel that border cell polarization requires a small GTPase, Rap1 (Ras-related protein 1), to collaborating with the Hippo signaling pathway to extend directional protrusions.

Loss of Rap1 severely hindered border cells from moving toward the destination. Rap1 hyperactivity disrupted the front/back polarity in border cells, in terms of evenly-distributed actin network and misoriented protrusions, and scattered border cells were observed more frequently too. By contrast, suppression of Rap1 activity made the clusters spin around with fewer protrusions, leading to impaired movement. In addition, individual border cells with higher Rap1 activity preferentially occupied the front of the migrating cluster, but conversely, Rap1-hypoactive ones lagged behind the other teammates. To uncover genes involved in Rap1-dependent group polarity, we carried out a

modifier screen and identified two core components of Hippo pathway, *hippo (hpo)* and *mob as tumor suppressor (mats)*. Down-regulation of either gene not only enhanced Rap1^{V12}-induced migration defect but also increased the protrusion number both in forward and backward directions. In Rap1 hyperactivity, *hpo* hemizygosity also significantly magnified the expression of Enable/Vasodilator-stimulated phosphoprotein (Ena/VASP) which promotes actin filament elongation. Moreover, pull-down analysis revealed stronger interaction of Rap1^{V12} and Hpo protein. Taken together, our finding suggests that at the leading edge of migrating border cells, activated Rap1 binds to Hpo to unleash its downstream effect on inhibiting Ena-F-actin dependent protrusions, which thereby promotes advancement of the migratory group.

110 Evolutionary drivers of rapid, episodic molecular evolution of *bag of marbles* (*bam*) in *Drosophila*: evaluating functional diversification and a conflict with *Wolbachia* *Jaclyn Bubnell*, Charles Aquadro. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

In *D. melanogaster*, *bag of marbles* (*bam*) acts as the master switch for germline stem cell (GSC) differentiation during oogenesis in females and plays a key role in regulating normal spermatogenesis in males. Interestingly, *bam* is rapidly evolving across the *Drosophila* genus. In *D. melanogaster* and *D. simulans, bam* shows a strong signature of positive selection with a strong burst of 60 nonsynonymous changes across its 443 amino acids in both lineages, but not in other lineages acorss the genus. To understand the functional consequences of positive selection at *bam,* our lab previously used site-specific interspecific transformation to express *D. simulans bam* in *D. melanogaster*. We observed female specific consequences of sequence divergence on fertility and GSC regulation between these species, but no effect on males. Our lab has hypothesized that the maternally inherited endosymbiotic bacteria *Wolbachia* may be a driver of positive selection at *bam* in *D. melanogaster* and *D. simulans*, and our lab's discovery that a genetic interaction between *Wolbachia* and a *bam* hypomorph results in partial rescue of female fertility in *D. melanogaster* is consistent with this hypothesis. However, there are several additional evolutionary pressures that could affect Bam protein sequence evolution. One possibility is that *bam's* function as the master switch for oogenesis is a novel function in the lineage leading to *D. melanogaster* and *D. simulans* and this function is now under positive selection in these species. We are currently testing the functional conservation of *bam*'s role in GSC differentiation in *Drosophila* by defining *bam's* function across diverse *Drosophila* species using CRISPR-Cas9 to create null alleles of *bam* in *D. simulans*, *D. yakuba* and *D. ananassae*. In bam null *D. yakuba* females, we have observed the same characteristic tumorous ovary phenotype and lack of developing oocytes observed in *D. melanogaster.* These preliminary results indicate that bam functions to regulate female GSC differentiation in *D. yakuba*. Further analysis of the *D. yakuba* bam null phenotype in females and males is underway. To further explore our hypothesis that *Wolbachia* has driven positive selection at *bam* in *D. melanogaster* and *D. simulans,* we are generating *bam* hypomorphic mutants in *D. simulans* and *D. yakuba* to evaluate *bam's* interaction with *Wolbachia* in these species.

111 Micromanagement of stem cell proliferation by the *Drosophila* testis niche Olga Valueva, Eugene Albert, *Christian Boekel*. CRTD, TU Dresden, Dresden, Germany.

Niches are traditionally thought of as signalling microenvironments that allow stem cells to adopt and maintain their fate. This definition implicitly contains the integration of multiple niche signals into a binary decision between stemness and differentiation. Using the somatic cyst stem cells (CySCs) of the *Drosophila* testis as a model system we find that niche signals instead directly impinge on specific, downstream stem cell properties that can therefore be genetically uncoupled from other aspects of stem cell behaviour.

CySCs are marked by the transcriptional regulator Zfh1, a shared target of the Hedgehog and Upd-Jak/STAT niche signalling cascades. Zfh1 overexpression is associated with increased CySC proliferation, while clones lacking Zfh1 fail to proliferate and are rapidly lost from the niche.

To identify the mechanism by which stem cell proliferation is regulated we have therefore mapped Zfh1 binding sites genome wide directly in the CySCs. The resulting set of potential target genes includes salvador and kibra, two upstream tumour suppressor genes of the Hippo/Warts/Yki pathway.

We demonstrate that Zfh-1 downregulates both genes *in vivo*. Yki thus becomes locally activated specifically in the Zfh-1 positive CySCs, which are, accordingly, the only cells in the somatic lineage that are actively dividing.

We furthermore show that Yki activation is necessary and sufficient for CySC proliferation. Clones of CySCs expressing activated Yki or lacking Kibra, Sav, or other upstream pathway components thus expand relative to controls.

Importantly, this Yki-mediated increase in proliferation does not affect the ability or propensity of the mutant clones to differentiate. The Upd and Hh niche signals thus "micromanage" stem cell proliferation through Zfh1 rather than inducing an overall stem cell fate.

We will discuss these results in the context of recent observations by other groups using the same and other model systems, and suggest that the currently accepted model of niche function may require a reappraisal.

112 Population Genomics of Parallel Cold Tolerance Evolution Within *Drosophila melanogaster* *John Pool*, Dylan Braun, Justin Lack. Laboratory of Genetics, University of Wisconsin - Madison, Madison, WI.

Drosophila melanogaster originated in tropical Africa before expanding into strikingly different temperate climates in Eurasia and beyond. Here, we show that elevated cold tolerance evolved not only as the species left Africa, but also twice within Africa, in the cool highlands of Ethiopia and South Africa. We sequenced more than 300 new inbred strain genomes from six natural population samples (encompassing a warm- and cold-adapted pair of populations for each origin of cold tolerance). Population genomic analysis, using our recently-described Population Branch Excess statistic (*PBE*) to assess evidence for local adaptation, then allowed us to assess evidence for genetically parallel evolution associated with recurrent cold tolerance adaptation. When *PBE* was applied to genomic windows (~4 kb), only limited evidence for parallel genetic differentiation of cold-tolerant populations was observed. In contrast, when we searched for single nucleotide polymorphisms (SNPs) with codirectional frequency change in two or three cold-adapted populations, strong genomic enrichments were observed from all comparisons. These findings could reflect an important role for selection on standing genetic variation leading to “soft sweeps”. An intronic SNP at the synaptic gene *Prosap* showed a particularly strong pattern of parallel allele frequency change; and more generally, proteins involved in neurotransmission were enriched as potential targets of parallel adaptation. The ability to study cold tolerance evolution in a parallel framework will enhance this classic study system for climate adaptation.

113 Genomics of adaptation coupled with a major dietary transition to herbivory in the Drosophilidae *Andrew Gloss*[1], Anna Nelson Dittrich[1], Noah Whiteman[2]. 1) University of Arizona, Tucson, AZ; 2) University of CA, Berkeley, CA.

The evolution of herbivory presents a paradox: herbivorous lineages are extremely successful evolutionarily, diversifying rapidly into new species, yet evolutionary transitions to herbivory are rare within insects. The difficulties of coping with abundant chemical toxins and the growth-limiting nutritional composition of living plant tissues are thought to be a major barrier preventing more frequent transitions to herbivory. We used comparative genomics, quantitiative genomics (e.g., GWAS), and enzyme biochemistry to uncover the genetic basis of adaptations enabling the evolution of herbivory in *Scaptomyza*, a genus nested within *Drosophila*. Through genome-wide association mapping and studies with mutant *Arabidopsis* plants, we found that mustard oils, the major defensive toxins in *Arabidopsis* and other mustard species used as hosts by herbivorous *Scaptomyza*, decrease *Scaptomyza* performance and shape feeding preference. Generalized detoxification enzymes (especially glutathione S-transferases) have undergone an elevated rate of gene duplication coupled with substrate specialization for mustard oils, revealed through genome sequencing and in *vitro* enzyme kinetics experiments. Other gene families that interact with dietary compounds, including growthlimiting sterols and ions, also exhibit more rapid evolution than in microbe-feeding *Drosophila* species. Through evolve and resequence experiments, we also found that *Scaptomyza* harbor extensive functional genetic variation within populations that enables rapid adaptation to host plant species. We propose this variation is maintained by fine-scale spatially varying selection, driven by the use of multiple sympatric host plant species in nature. Overall, we find that antagonistic interactions with host plants drive gene family remodeling and maintain genetic variation in *Scaptomyza*.

114 The genetic basis of rapid adaptive shifts in pigmentation over seasonal time scales *Alan Bergland*[1,3], Subhash Rajpurohit[2], Dmitri Petrov[3], Paul Schmidt[2]. 1) Dept. of Biology, University of Virginia, Charlottesville, VA; 2) Dept. of Biology, University of Pennsylvania, Philadelphia, PA; 3) Dept. of Biology, Stanford University, Stanford, CA.

Fly populations living in temperate environments rapidly adapt to seasonal shifts in selection pressures. These adaptive dynamics ultimately lead to oscillations in phenotype and genotype over seasonal timescales, and such shifts have been observed in various morphological and life-history traits as well as at hundreds of SNPs genome-wide. However, to date, we have little knowledge of how alleles underlying genetic variation in seasonally selected traits change in frequency through time. To further our understanding of the genetic dynamics of rapid adaptation, we examined pigmentation variation over seasonal time scales within and among North American populations. First, we show that flies collected in early spring are genetically darker than those collected in fall over four years of consecutive in a focal Pennsylvanian population. Seasonal shifts in pigmentation reflect latitudinal variation in pigmentation, whereby high latitude populations are genetically darker than equatorial ones. Next, we examine spatial and temporal variation in alleles underlying pigmentation variation. We show that top ranked candidate SNPs underlying pigmentation among multiple *D. melanogaster* mapping populations do not explain spatial and temporal variation in pigmentation: at the majority of these SNPs the dark allele is at higher frequency in phenotypically lighter, equatorial populations - patterns which we show are consistent with the recent dual colonization of North America by European and African populations. Top candidate SNPs also show no appreciable change in frequency among seasons. To explain observed seasonal and latitudinal shifts in pigmentation, we identified alternative classes of polymorphisms. These polymorphisms show concordant shifts in allele frequency among populations sampled along the latitudinal cline and show semi-predictable (~75%) and dramatic changes in allele frequency among seasons over

multiple years in the same focal Pennsylvanian population. Taken together, our results suggest that minor affect alleles largely contribute to rapid evolution and that cyclic adaptation in quantitative traits is partially predictable at the genetic level.

115 Rapid evolution of learning in natural populations of *Drosophila melanogaster* *Emily Behrman*[1,2], Tadeusz Kawecki[2], Paul Schmidt[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) University of Lausanne, Lausanne, Switzerland.

Understanding the evolution of behavior is fundamental because behavior contributes both directly and indirectly to an organisms' survival or reproductive success. Organisms use learning in many aspects of their biology (e.g., foraging, mating and predator avoidance) to modify their behavior based on previous experiences to increase survival or reproduction. Learning is assumed to evolve if the benefits of learning are strong and increase fitness despite potential costs. Despite the significance of learning, it is unclear how rapidly this complex behavior evolves. Here, we investigate the rate of learning evolution in natural populations of *Drosophila melanogaster*. We assess learning in natural populations collected across seasonal time using classical conditioning methods in the laboratory complemented with a natural field environment. We show that learning evolves rapidly from spring to fall over replicate years. The differences in learning across seasonal time are associated with seasonally cycling alleles in the RNA binding protein *couch potato* (*cpo*)*.* The differences in learning ability across populations suggest that the benefits of learning drive its evolution but also imply certain costs or else all individuals would learn maximally well. We test the hypothesis that the differential expression of *couch potato* regulates a fitness trade-off between learning and reproductive output. Together, we show that learning is among a suite of fitness-associated traits that evolve rapidly in natural populations of *D. melanogaster* across seasonal time and *couch potato* facilitates trade-offs between learning and reproductive output

116 HP1 gene family diversification suggests recurrent innovation in paternal chromosome packaging across 250 million years of Diptera evolution *Quentin Helleu*, Mia Levine. Department of Biology, University of Pennsylvania, Philadelphia, PA.

Heterochromatin is the gene-poor, repeat-rich, and late-replicating genome compartment concentrated around centromeres and telomeres. Heterochromatin packaging is required for strictly conserved processes like chromosome segregation and genome stability, but accumulating evidence suggests that some heterochromatin proteins are wildly unconserved. The *Drosophila* Heterochromatin Protein 1 (HP1) gene family is exemplary. HP1 members are defined by a chromo domain that interacts with histone tail modifications and a chromo shadow domain that mediates protein-protein interactions. A previous *Drosophila*-focused phylogenomic analysis revealed abundant lineage-specific, rapidly evolving, and testis-enriched HP1 members. This pattern of male-specific HP1 diversification, in combination with subsequent functional analyses of two such HP1s, revealed that paternal heterochromatin packaging requires recurrent innovation in *Drosophila*. Given that heterochromatic sequence and structure evolve exceptionally fast across all eukaryotes, we hypothesized that HP1 genes recurrently evolve to package paternal heterochromatin beyond the *Drosophila* genus. To test this prediction, we conducted an HP1 phylogenomic analysis across 250 million years of Diptera evolution. This insect order spans remarkable structural and sequence diversity in the heterochromatic compartment, including chromosomal rearrangements, repeat-driven genome size expansion/reduction (from 99 to 1 343 Mbp), B chromosomes, transposable element invasions, and transitions from heterochromatic to homomorphic sex chromosomes. Using BLAST, phylogenetics, and synteny analysis, we discovered widespread HP1 gene turnover: 133 gene gain events and 23 gene loss events span 74 Diptera genomes. In contrast to this dramatic HP1 gene turnover, we uncovered only two gain and two loss events across four other chromo domain-encoding gene families. Not all Diptera clades encode many lineage-restricted HP1s. Indeed, HP1 paralog numbers range from 2 (e.g. in *Anopheles gambiae*) to 36 (in *Mayetiola destructor*). These striking differences in gene family size suggest the possibility that some clades harbor a less dynamic heterochromatic compartment or, more likely, that other gene families are recruited to package dynamically evolving heterochromatin. Furthermore, analysis of tissue-specific transcriptome datasets and our own gene-specific qPCR assays across dissected somatic and germline tissues revealed that most lineage-restricted HP1s across Diptera are enriched in the testis. Our data implicate spermatogenesis as a key developmental process driving heterochromatin protein evolution, possibly related to selfish genetic elements that hijack paternal chromosome transmission.

117 Recurrent gene duplication leads to diverse repertoires of centromeric histones in Drosophila species *Lisa Kursel*[1,2], Harmit Malik[1,3]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA; 3) HHMI.

Despite their essential role in the process of chromosome segregation in most eukaryotes, centromeric histones show remarkable evolutionary lability. Not only have they been lost in multiple insect lineages, but they have also undergone gene duplication in multiple plant lineages. Based on detailed study of a handful of model organisms including *Drosophila melanogaster*, centromeric histone duplication is considered to be rare in animals. Using a

detailed phylogenomic study, we find that *Cid*, the centromeric histone gene, has undergone four independent gene duplications during *Drosophila* evolution. We find duplicate *Cid* genes in *D. eugracilis* (*Cid2*), in the *montium* species subgroup (*Cid3, Cid4*) and in the entire *Drosophila* subgenus (*Cid5)*. We show that Cid3, Cid4, Cid5 all localize to centromeres in their respective species. Some *Cid* duplicates are primarily expressed in the male germline. With rare exceptions, *Cid* duplicates have been strictly retained after birth, suggesting that they perform non-redundant centromeric functions, independent from the ancestral *Cid*. Indeed, each duplicate encodes a distinct N-terminal tail, which may provide the basis for distinct protein-protein interactions. In addition, we show some *Cid* duplicates evolve under positive selection whereas others do not. Finally, we have used Cid5-specific antibodies to determine that Cid5 functions post-meiosis and localizes to foci in the nuclei of developing spermatids in *D. virilis*. These results suggest that Cid5 has acquired a specialized centromeric role in the male germline. Taken together, our results support the hypothesis that *Drosophila* Cid duplicates have subfunctionalized. Thus, these gene duplications provide an unprecedented opportunity to dissect the multiple roles of centromeric histones.

118 Dynamics of a natural P-element invasion in experimentally evolving populations of *D. simulans* *Robert Kofler*[1], Kirsten Andre Senti[1], Viola Nolte[1], Ray Tobler[1,2], Christian Schlötterer[1]. 1) Instititute of Population Genetics/VetMedUni Wien/Veterinaerplatz 1/1210 Wien; 2) Australian Centre for Ancient DNA/University of Adelaide.

The P-element, one of the best understood eukaryotic transposable elements (TEs) recently invaded natural *D. simulans* populations. We captured a natural *D. simulans* population from Florida at an early stage of the invasion and set up a replicated experimental evolution study in hot and cold environments. This opens the unprecedented opportunity to study a natural invasion of a TE with the aid of high throughput sequencing technologies in replicated populations evolving in different environments. We show that in all replicate populations of a given environment the P-element rapidly spreads with a remarkable consistency. In the hot environment P-element copy numbers increased 16-fold up to generation 20 and attained a stable copy number of about 30 per haploid genome. No further increase could be noted during the next 40 generations of experimental evolution. By contrast, at cold conditions the speed of the invasion is much slower, the P-element multiplied 4-fold by generation 30. Interestingly, the P-element invasion only results in a modest reactivation of resident TE families in D. simulans. The levelling out of the P-element invasion at hot conditions could be due to i) a balance between transposition events and negative selection against TE insertions ii) non-autonomous truncated copies of the P-element, which have been shown to down-regulate transposition activity and iii) piRNAs. RNA-seq and small RNA-seq analysis argues that the dominant factor containing the spread of the P-element is the emergence of piRNAs complementary to the P-element.

119 *C*hitinase-like proteins disrupt tube morphogenesis *Sandra Zimmerman*, Anne Sustar, Genneifer Merrihew, Liesl Strand, Michael MacCoss, Celeste Berg. Department of Gemone Sciences, University of Washington, Seattle, WA.

Chitin is a major constituent of the exoskeleton of insects and the second most abundant natural biopolymer on earth, second only to cellulose. Although mammals do not synthesize chitin, they synthesize chitinases and enzymatically inactive chitinase-like proteins (CLPs). Elevated levels of human chitinases and CLPs are associated with chronic inflammatory diseases and several cancers, often correlating with poor prognosis. Normally involved in immune responses and wound healing, upregulation of these family 18 glycosyl hydrolases (GH18 family) can promote disease progression by remodeling tissue, activating signaling cascades, stimulating proliferation and migration, and by regulating adhesion. Nevertheless, there is scant knowledge of their function or what mutations may influence their expression. We identified several orthologs of human CLPs, members of the *Drosophila* family of Imaginal disc growth factors (Idgfs), in a proteomics analysis designed to discover regulators of dorsal-appendage tube morphogenesis in the fly ovary. Phylogenetic analyses suggest that Idgfs and CLPs arose by duplication of ancient chitinases that existed in a common ancestor of fungi and animals. A conserved mutation rendered them inactive as chitinases, allowing them to evolve new functions. Comparative mass spectrometry revealed increased abundance of four of the six Idgfs as a downstream consequence of loss of function in the SOX transcription factor Bullwinkle (Bwk). *bwk* mutations cause severe defects in morphogenesis of the epithelial tubes that form and secrete the dorsal appendages, eggshell structures that facilitate gas exchange with the embryo. Using RNAi, overexpression constructs, and newly generated null alleles, we show that dysregulation of Idgfs disrupts tube formation of the dorsal-appendage-forming tubes, with overexpression being particularly deleterious. The tubes fail to close and do not properly elongate, mimicking defects caused by *bwk* mutations. Understanding how Idgfs function in a *Drosophila* model of tissue morphogenesis will provide insight into the role of their human orthologs in tissue remodeling, inflammation, and injury. Due to their role as disease biomarkers and potential drug targets, there is a critical need for understanding how CLPs function.

120 Organ sculpting by patterned extracellular matrix stiffness *Justin Crest*[1], Alba Diz Munoz[3], Dong Yuan Chen[1], Dan Fletcher[2], David Bilder[1]. 1) Dept Mol and Cell Biology, Univ California, Berkeley, CA; 2) Dept of

Bioengineering, Univ California, Berkeley, CA; 3) Department of Biophysics, EMBL, Heidelburg, Germany.
The precise forms of organs are ultimately shaped by spatial variation in net physical forces. A central question of morphogenesis is how this mechanical anisotropy is generated within an organ's components. Elegant studies have revealed conserved mechanisms that drive tissue elongation via cell-intrinsic and polarized remodeling of adherens junctions. In contrast, the capacities of extracellular properties to confer specific shapes are poorly understood. Here we show that organs can be sculpted by patterning anisotropic resistance within their ECM, rather than anisotropic forces within their cells. Through analyzing the elongation of the developing Drosophila egg using direct biophysical measurements, we document robust mechanical anisotropy in the ECM-based basement membrane (BM) but not in the underlying epithelium. Atomic force microscopy (AFM) on *in vivo* BM reveals a symmetric stiffness gradient that develops along the anterior-posterior axis, while elongation-defective mutants retain a uniformly soft BM. Genetic manipulation of BM components shows that the BM is instructive for tissue elongation and that the determinant is relative rather than absolute stiffness, creating differential resistance to isotropic tissue expansion. The stiffness gradient requires morphogen-like signaling to regulate A-P BM incorporation, as well as planar-polarized organization to homogenize circumferential stiffness. Our results demonstrate how fine mechanical patterning in the ECM can work in tandem with cells to shape an organ.

121 Semaphorin-Plexin signaling regulates stress fiber dynamics during epithelial migration *Claire Stevenson*[1], William Menegas[2], Sally Horne-Badovinac[1,3]. 1) Committee on Development, Regeneration and Stem Cell Biology, University of Chicago, Chicago, IL; 2) Department of Molecular Genetics and Cellular Biology, Harvard University, Cambridge, MA; 3) Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL.
Collective migration of cells within an epithelial sheet is central to many biological processes including morphogenesis, wound healing and tumor invasion. Similar to individually migrating cells, each epithelial cell extends actin-rich protrusions from its leading edge and disassembles actin-based stress fibers at its trailing edge. Unlike individually migrating cells, however, migrating epithelial cells must coordinate these behaviors with their neighbors. How this coordination is achieved is largely unknown. Studying the rotational motion of the follicular epithelium, we have identified a transmembrane Semaphorin (Sema-5c) as a novel regulator of this process. Semaphorins are signaling proteins that activate Plexin receptors, and are best known for their roles in axon guidance. Among the five *Drosophila* Semaphorins, Sema-5c is the only family member with a direct mammalian homolog, yet virtually nothing is known about its function. We have found that Sema-5c promotes epithelial migration. Moreover, loss of Plexin-A (PlexA) phenocopies loss of Sema-5c, suggesting that PlexA may transduce the Sema-5c signal. PlexA shows a planar polarized distribution at the basal epithelial surface, such that it is enriched along leading-trailing cell-cell interfaces. To understand how Sema-5c - PlexA signaling promotes epithelial migration, we have initially focused on the PlexA effector Mical, which preferentially disassembles bundled F-actin in other cell types. Mical knockdown selectively increases actin levels in the stress fibers at the basal surface of each follicle cell, and Mical overexpression eliminates these structures. We have further found that Mical localizes in a punctate pattern at the trailing ends of individual stress fibers and that PlexA is required for this localization. Altogether, our data suggest that a novel Sema-5c - Plex-A - Mical signaling mechanism promotes epithelial migration by locally regulating stress fiber turnover at the trailing edge of each cell.

122 The epithelial-specific zinc finger transcription factor Ichor is essential for seamless tube morphogenesis in the *Drosophila* tracheal system *Jeff Rosa*, Amin Ghabrial. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
Branched tubular organs carry out critical homeostatic functions, including the transport of nutrients and gases and secretion of fluids. Comprised of apicobasally polarized cells, tubes organize their apical domains into a luminal compartment. However, the processes regulating the morphogenesis and functional maturation of these luminal compartments are poorly understood. Luminal compartments can be sealed by intercellular junctions, as in multicellular tubes or form within a single cell and be devoid of cell junctions, as in unicellular seamless tubes. Seamless tubes form in vertebrates within specialized endothelial tip cells that mediate vascular anastomosis during sprouting angiogenesis, as well as in specialized tip cells, called terminal cells, in the *Drosophila* tracheal (respiratory) system. Despite the conservation of seamless tubes in metazoans, the factors regulating their morphogenesis are incompletely understood. From a forward genetic screen in larval terminal cells, we have identified a novel zinc finger protein, Ichor (Ich), required for the functional maturation of seamless tubes, culminating in gas-filling. Though initial seamless tube formation is *ich*-independent, *ich* terminal cells later exhibit cystic, discontinuous seamless tubes, along with the accumulation of vacuole-like apical membrane structures instead of a proper lumen, indicating that defects in apical membrane morphogenesis underlie the lumen maturation defects. We show that Ichor functions as a transcriptional activator to regulate apical membrane morphogenesis in the terminal cell. We further show that *ich* is expressed specifically in cuticle-secreting epithelia, suggesting a role for *ich* in chitin-based apical extracellular matrix (aECM) biogenesis. Consistent with a role for aECM biogenesis in seamless tube morphogenesis, we find that

another mutant, *short-of-breath*, disrupts Krotzkopf Verkerht/Chitin Synthase-1 activity and exhibits apical membrane defects similar to *ich* mutants. These findings suggest that the morphogenesis and functional maturation of seamless lumens involves the activation of a transcriptional program mediating aECM assembly.

123 Post-transcriptional downregulation of Bazooka-Par3 downstream of Snail in epithelial-mesenchymal transition *Joan Y. Lee*[1,2], Mo Weng[1,2], Eric Wieschaus[1,2]. 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) HHMI, Princeton, NJ.

The Snail transcriptional factor, a master driver of epithelial-mesenchymal transition, has long been studied as a transcriptional repressor of polarity genes and adherens junction components. Our previous data demonstrated that Snail expression in the mesoderm of the gastrulating Drosophila embryo is necessary and sufficient to downregulate adherens junctions at the protein level without affecting other E-Cadherin pools, and that such junction loss may take place through downregulation of the polarity protein Par3/Bazooka (Baz). Consistent with such a role, we found that although levels of subapically localized Baz are comparable in the mesoderm and the ectoderm during early cellularization, mesoderm Baz levels gradually decrease as Snail expression increases during mid- to late cellularization. The region of decreased Baz levels corresponds with the Snail expression domain and such downregulation is absent in Snail mutants. Ectopic expression of Snail in non-mesodermal tissues is sufficient to downregulate subapical Baz independently of junctions.

We found that embryos lacking the X chromosome fail to downregulate Baz. Using a series of translocation and duplication chromosomes, we have identified a small genomic region that, when deleted, increases mesoderm Baz to levels comparable to ectoderm, even in embryos lacking the zygotic Baz locus. A genomic fragment covering the region restores normal downregulation. The same region also affects the downregulation of junctional components such as Armadillo/ß-catenin. Our results suggest that zygotic expression of a single gene on the X chromosome may mediate Snail-dependent downregulation of both Baz and junctions. This junction loss ultimately leads to an epithelial-mesenchymal transition of the mesoderm and has been shown previously to be sensitive to mechanical tension applied. Identification of the gene or genes mediating this junction loss may therefore provide a platform to understand the mechanosensitive regulation of junctions.

124 Dynamin regulates actin cytoskeletal organization during cell-cell fusion *Nathalie Gerassimov*[1], SangJoon Kim[1], Arnaldo Mercado-Perez[1], Tiffaney Tran[1], Delgermaa Luvsanjav[1], Elizabeth Chen[1,2]. 1) Cell Biology, Johns Hopkins Medical School, Baltimore, MD; 2) Cell Biology, UTSouthwestern Medical Center, Dallas, Tx.

Myoblast fusion leads to the formation of multinucleated muscle fibers and is essential for muscle development and regeneration. *Drosophila* embryonic muscle development has been an instrumental *in vivo* system to uncover evolutionarily conserved cellular and molecular mechanisms of myoblast fusion. Work from our lab has shown that myoblast fusion is promoted by a cell type-specific, F-actin-enriched podosome-like structure (PLS) that invades the apposing fusion partner with multiple finger-like protrusions at the fusogenic synapse. We have recently identified that the conserved large GTPase Dynamin (Dyn), best known for its function in endocytosis, is a critical component of myoblast fusion *in vivo*. Interference with Dyn function during myoblast fusion using temperature-sensitive alleles, *shibire^{ts}*, leads to a severe myoblast fusion defect, which can be rescued by overexpressing wild-type Dyn. Furthermore, RNAi knockdown of Dyn in cultured cells that are induced to fuse also results in a fusion defect, suggesting a general role for Dyn in cell-cell fusion. We show that Dyn is enriched within the F-actin foci at the fusogenic synapse in wild-type embryos and that the F-actin foci exhibit abnormal morphology in *shi^{ts}* mutant embryos at restrictive temperature, indicating a function of Dyn in organizing these actin-enriched structures. Interestingly, electron microscopy analysis revealed no endocytic vesicles at the fusogenic synapse where Dyn is enriched in wild-type embryos and no collared pits are observed at the fusogenic synapse in *shi^{ts}* mutant embryos at restrictive temperature, suggesting that endocytosis may not play a direct role in myoblast fusion. Together, our findings strongly support a novel endocytosis-independent function of Dyn in regulating F-actin organization during cell-cell fusion.

125 Systematic transcriptome analysis of flight muscle development reveals that Spalt major regulates a biphasic mode of sarcomere assembly *M.L. Spletter*[1,2], C. Barz[1], A. Yeroslaviz[1], S.B. Lemke[1], X. Zhang[1], B.H. Habermann[1,3], F. Schnorrer[1,3]. 1) Max Planck Institute for Biochemistry, Martinsried, Germany; 2) Biomedical Center, Physiological Chemistry, Martinsried, Germany; 3) IBDM, Marseille, France.

Muscles must organize a pseudo-crystalline array of actin, myosin and titin filaments to build functional sarcomeres, the mini-machines that drive muscle contraction. To understand how sarcomerogenesis is regulated during development, we performed mRNA-Seq at 8 time points spanning the entire development of the *Drosophila* indirect flight muscles. On the genome-wide level, we identify at least 40 distinct developmental expression profiles, many of which match characterized indicator gene profiles and correspond with major transition points in muscle development, such as myoblast fusion and the initiation of sarcomerogenesis. Strikingly, we find a major transcriptional shift in gene expression after the initiation of sarcomerogenesis and genes upregulated at this

transition are strongly enriched for sarcomeric components. We show that this shift corresponds to the process of sarcomere maturation: an immature, contractile sarcomeric array is assembled before 48h APF and then subsequently grows both in sarcomere length and width by incorporation of sarcomeric components upregulated after 48h APF to assemble a fully functional pseudo-crystalline sarcomere capable of supporting flight. We identify Spalt major (Salm) as an important regulator for this second phase of sarcomere maturation. Stage-specific loss of *salm* in mid-development partially blocks transcriptional upregulation and consequently results in sarcomere growth defects. Importantly, loss of one Salm target, the titin related molecule Stretchin-Mlck, results in developmental sarcomere overgrowth leading to muscle hypercontraction and atrophy in the adult. Our data suggest a two-step model of sarcomere development in which Salm plays a key role in inducing genes essential for sarcomere maturation, such as Stretchin-Mlck, in addition to its established role in initiating flight muscle identity.

126 Anastasis, a conserved cell survival strategy under stress *Gongping Sun*[1], Xun Ding[1,2], Rebecca Cheng[1], Denise Montell[1]. 1) Molecular, cellular, and developmental biology, University of California, Santa Barbara, Santa Barbara, CA; 2) Department of biological chemistry, Center for cell dynamics, John Hopkins University School of Medicine, Baltimore, MD.

Caspase-3 carries out the executioner phase of apoptosis, however under special circumstances, cells can survive its activity. We designed a system, CasExpress, which drives fluorescent protein expression, transiently or permanently, in cells that survive caspase-3 activation in Drosophila. Using CasExpress, we discovered widespread survival of caspase-3 activity during development. Previously our lab reported in mammalian cells can survive caspase 3 activation following transient apoptotic stimuli, a process named anastasis. To figure out if in vivo cells can survive caspase activation after exposing to apoptotic stress, we treated Drosophila larvae with heat shock and X-ray irradiation. Heat shock induces mild apoptosis while irradiation results in massive apoptosis. We found a lot of cells with CasExpress activation after heat shock and irradiation, indicating these cells surviving apoptotic caspase activation. Furthermore, we found anastatic cells are required for wing regeneration. Our study provides the evidence of the biological necessity of anastasis, a conserved cell survival process, and unveils a new role of caspase 3 in tissue regeneration.

127 Stretch Follicle Cells Utilize Lysosomal Proteins to Eliminate Nurse Cells by Phagoptosis *Albert Mondragon*, Anthony Ortega, Yuanhang Zhang, Kim McCall. Boston University, Boston, MA.

Phagoptosis is the most prevalent form of cell death in the human body. In phagoptosis, one cell utilizes phagocytosis machinery to kill another cell that would continue living. We have recently shown that ovarian nurse cells die by phagoptosis, providing a powerful model for this poorly understood type of cell death. In the Drosophila ovary there are 15 nurse cells that support the oocyte throughout development. The nurse cells and oocyte are surrounded by a layer of follicle cells. In late oogenesis the nurse cells dump their cytoplasmic contents into the oocyte and are encompassed by a subset of follicle cells called stretch follicle cells. We have demonstrated that the stretch follicle cells utilize phagocytosis machinery (Draper, Ced-12, integrins and the JNK pathway) to promote nurse cell death; however, the exact mechanism remained elusive. Through live imaging, an *in vivo* engulfment detector, and an RNAi screen, we have determined that the stretch follicle cells utilize lysosomal machinery to acidify and breakdown the nurse cells. Live imaging of egg chambers with probes and GFP fusion proteins as well as the *in vivo* engulfment detector has demonstrated that nurse cells are not engulfed piece-wise, but acidified entirely. Interestingly, MITF and V-ATPases are required in the stretch follicle cells for nurse cell acidification. V-ATPases become enriched in stretch follicle cells in late oogenesis and localize to the plasma membranes of the stretch follicle cells to acidify the nurse cells that they surround. Altogether this work further characterizes a novel form of cell death and illustrates the importance of lysosomal components acting through a non-autonomous mechanism to control the death of neighboring cells.

128 COPI–Arf1–lipolysis pathway regulates normal and transformed stem cells survival in adult *Drosophila* *Shree Ram Singh*, Xiankun Zeng, Jiangsha Zhao, Ying Liu, Gerald Hou, Hanhan Liu, Steven Hou. Basic Research Laboratory, National Cancer Institute, Frederick, MD.

Stem cells can be found in both normal adult organs and cancers. These cells are usually resistant to cytotoxic conditions and may be responsible for tumor recurrence after initial chemotherapy. Although the biology behind this therapy resistance is still poorly understood, recent studies suggest that a small population of cells within a tumor, called cancer stem cells (CSCs), may be responsible for tumor dormancy, relapse, and the eventual death of most cancer patients.

Previous studies have demonstrated the relevance of oncogenes and tumor suppressors in controlling intestinal stem cell-mediated tumorigenesis in *Drosophila*. To explore the molecular mechanism behind the therapy resistance of CSCs, we investigated the death of stem cell in the adult digestive system of *Drosophila*. In this system, three organs, the posterior midgut, the hindgut, and the Malpighian tubules (MTs), meet and join at the junction of the posterior midgut and hindgut. Stem cells in these organs exhibit different degrees of quiescence. The intestinal stem

cells (ISCs), located in the posterior midgut, divide once every 24 hours; the renal and nephric stem cells (RNSCs), located in the MTs, divide about once a week; and the quiescent hindgut intestinal stem cells (HISCs), found at the midgut/hindgut junction, divide only during stress-induced tissue repair. ISCs and RNSCs can be transformed to produce neoplastic-like tumors: ISCs by knocking down their Notch (N) activity, and RNSCs by forcing their expression of a constitutively activated for of Ras oncogene. These transformed stem cells thus resemble CSCs.

Here we found that both normal and transformed stem cells in adult *Drosophila* are resistant to pro-apoptotic gene-induced cell death, but knocking down the COPI/Arf1 complex specifically kills normal and transformed stem cells through necrosis, by attenuating the lipolysis pathway**,** but spared differentiated cells. The dying stem cells were engulfed by neighboring differentiated cells through a Draper-Mbc/Rac1-JNK-dependent autophagy pathway. Furthermore, Arf1 inhibitors reduced CSCs in human cancer cell lines. Our data suggest that, like hibernating animals, normal and transformed stem cells may rely on lipid reserves for their energy supply, and blocking lipolysis can starve them to death. This finding will help to develop novel therapies to eliminate CSCs in human cancers.

129 Src-MAPK, Hippo and TGFB signaling cooperatively regulate cytokine production in enterocytes upon bacterial infection *Philip Houtz*[1], Alessandro Bonfini[1], Xi Liu[1], Jonathan Revah[1], Aurélien Guillou[1], Korneel Hens[2], Bart Deplancke[3], Yu-Chen Tsai[4], Nicolas Buchon[1]. 1) Entomology Dept, Cornell University, Ithaca, NY; 2) Center for Neural Circuits and Behavior, The University of Oxford, Tinsley Building, Mansfield Road, Oxford, OX1 3SR, UK; 3) Laboratory of Systems Biology and Genetics (LSBG). Ecole Polytechnique Federale de Lausanne. Station 19, AAB 145. CH-1015, Lausanne, Switzerland; 4) Department of Life Science and Life Science Center, Tunghai University, Taichung, Taiwan, Republic of China.

Cytokine production in the digestive tract is responsible for coordinating protective epithelial regeneration and immune responses. However, it remains unclear how cytokine production is transcriptionally regulated in the gut epithelium. In *Drosophila*, Upd3 is the major signaling protein induced in enterocytes and enteroblasts upon oral infection, and activates the JAK-STAT pathway to promote intestinal stem cell (ISC) dependent tissue repair. To date, the genetic network directing *upd3* transcription remains largely uncharacterized. We have identified the key transcription factors that control *upd3* enhancer activity during an enteric infection. Through functional genetic screening, bioinformatic analyses and yeast one-hybrid screening, we determined that the transcription factors Sd, Mad, D-Fos, and Snail are principal regulators of *upd3* expression. In addition, our study demonstrates that *upd3* transcription in the midgut is cooperatively regulated by multiple avenues, including the Src-Raf-DSOR1-ERK, p38 MAPK, Hippo and TGFb/DPP pathways. These gene networks, which have previously been shown to control ISC proliferation cell-autonomously, thus act in enterocytes to promote tissue turnover indirectly, through the regulation of *upd3* transcription.

130 Olfaction mediated neuronal control for immune competency in *Drosophila* blood cells via GABA-shunt *Sukanya Madhwal*, Manish Joshi, Ankita Kapoor, Pirjzada M Rehman, Tina Mukherjee. inStem, NCBS, Bangalore, India.

The development of a competent immune system establishes the core efficiency of the host's immune response against infections. While much attention is focused on deciphering the influence of infections in cellular immune response, programs that maintain immune competency during normal hematopoietic development remain poorly understood. *Drosophila* larvae infected with parasitic wasps, *L.boulardi* initiate a process of cellular immune response that is marked by the appearance of large flat cells called "lamellocytes". These cells are however rarely found in the animal and their appearance is a hallmark of cellular immune response. Our recent findings reveal an important role of odor sensing and olfactory signaling in capacitating hematopoietic progenitor cells to generate lamellocytes upon wasp infections. Olfaction mediated systemic release of GABA from brain neurosecretory cells is internalized by blood progenitor cells and its subsequent metabolism into succinate via the GABA shunt pathway primes immune progenitors to maintain competency to generate effective lamellocytes. The use of GABA-shunt by blood progenitor cells is specific to generating these specialized cells during infections and does not perturb the normal hematopoietic development of the animals. Importantly, larvae reared in pathogenic odorants during development display elevated GABA and when infected respond with further rapidity. Thus our data reveals the developmental importance of environmental odor sensing as a critical regulator of immune progenitor competency via modulating systemic cue GABA. Overall, this study highlights the importance of sensory neuronal cues in modulating the metabolic landscape of immune cells for a competent and an effective immune system.

131 A new family of GTPases from virulence-linked extracellular microvesicles of a Drosophila generalist parasite *M. Heavner*[1], J. Ramroop[1], B. Wey[1], J. Crissman[2], E. Miller[3], S. Singh[1], S. Govind[1]. 1) The Graduate Center, CUNY; 2) Columbia Univ, NY; 3) Cambridge Univ, UK.

Specialized virulence and their oppositional host defense factors shape the biological arms race. The parasitic wasp *Leptopilina heterotoma (Lh)* produces 300 nm virus-like particles (VLPs) and successfully infects many fly species. During infection *Lh* VLPs obliterate hemocytes and thus suppress host defense. Unlike viruses, VLPs are

biosynthesized in steps occurring inside and outside venom gland cells. They lack viral coat proteins and do not replicate in wasp or fly cells. We have shown that *Lh* VLPs are microvesicular and carry immune modulating, cell killing, and novel proteins.

A major component of VLPs, but previously unannotated family of GTPases will be presented. This family is absent in the non-cell killing VLPs of *L. boulardi*, a sister wasp with a host range limited to the *D. melanogaster* species group. Using computational analyses we have characterized 3 small (SmGTPases) and 5 large (LgGTPases) family members with key residues for GTP hydrolysis, high similarity in their Ras-like N-termini, and C-termini coiled-coils specific to the large GTPases. Five of the 8 members possess prokaryotic domain annotations.

Individual expression and imaging in yeast of a small and large representative GTPase (SmGTPase01 and LgGTPase01) shows cytoplasmic localization with focal concentration around an over-abundant population of small vacuole-like organelles. Genome-wide synthetic lethality screens with SmGTPase01 in yeast showed growth repression in strains compromised in intracellular trafficking, mitochondrial, and vacuolar (analogous to lysosomes) functions. SmGTPase01 and LgGTPase01 co-expression enhances dysfunction within the endomembrane system as compared to SmGTPase01 expression alone.

We hypothesized that GTPase family members cooperatively stress intracellular transport and membrane reserves during retrograde transport of VLPs by host macrophages, the target host cells. Our fly assays for VLP function using subcellular compartment markers and immune/cell death assays will greatly clarify the impact of VLPs on host cell physiology and their immune suppressive mechanisms. VLPs constitute a unique class of extracellular microvesicle-like organelles that shape genetic structures of insect communities. Our findings are relevant to organelle biogenesis and function, as well as to the understanding of the molecular basis of natural host-parasite interactions.

132 PGRP-SD is an extracellular pattern recognition receptor that enhances peptidoglycan-mediated activation of the *Drosophila* Imd pathway *Igor Iatsenko*[1], Shu Kondo[2], Dominique Mengin-Lecreulx[3], Bruno Lemaitre[1]. 1) Global Health Institute, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; 2) National Institute of Genetics, Mishima, Japan; 3) Institute for Integrative Biology of the Cell (I2BC), France.

Activation of the innate immune response in Metazoans is initiated through the recognition of microbes by host pattern recognition receptors (PRRs). *Drosophila* peptidoglycan recognition proteins (PGRPs) function as pattern recognition receptors upstream of Toll and Imd pathways. In *Drosophila*, diaminopimelic acid-containing (DAP) peptidoglycan from Gram-negative bacteria is detected by the transmembrane receptor PGRP-LC, and by the intracellular receptor PGRP-LE, leading to activation of Imd pathway. Toll pathway activation requires recognition of Gram-positive Lys-type peptidoglycan by PGRP-SA and GNBP1. For a long time, PGRP-SD was considered as a pattern recognition receptor for Gram-positive bacteria. However, crystal structure of PGRP-SD revealed its affinity to DAP-type peptidoglycan, questioning the role of PGRP-SD in the Toll pathway. Using newly generated *PGRP-SD* mutants, we showed that PGRP-SD acts upstream of PGRP-LC as an extracellular receptor, to enhance peptidoglycan-mediated activation of Imd signalling. Consistent with this, *PGRP-SD* mutants exhibited impaired Imd pathway activation and increased susceptibility to DAP-type bacteria. We show that PGRP-SD co-immunoprecipitates with PGRP-LCx in the presence of peptidoglycan and enhances peptidoglycan-mediated activation of Imd pathway in a PGRP-LC-dependent manner. Additionally, we found that PGRP-SD promotes peptidoglycan concentration at the proximity of the cell membrane and hence promotes signalling. Moreover, PGRP-SD antagonizes the action of PGRP-LB, an extracellular negative regulator to fine-tune the intensity of the immune response. Furthermore, we found essential role for PGRP-SD in the gut immune response to pathogens as well as in the tolerance to microbiota. These data reveal that *Drosophila* PGRP-SD functions as an extracellular receptor similar to mammalian CD14, and demonstrate that, comparable to lipopolysaccharide sensing in mammals, *Drosophila* relies on both intra- and extracellular receptors for the detection of bacteria.

133 Phosphorylation of Threonine 11 in Histone H3 marks insulator elements and counteracts *Polycomb* dependent H3 Lysine 27 methylation *Alf Herzig*[1], Ho-Ryun Chung[2], Alisa Fuchs[2], Katharina Kawall[1]. 1) Cellular Microbiolgy, Max Planck Institute for Infection Biology, Berlin, Berlin, Germany; 2) Computational Epigenomics, Max Planck Institute for Molecular Genetics, Berlin, Berlin, Germany.

Polycomb group proteins are evolutionary conserved regulators of developmental gene expression in metazoans. Enhancer of zeste (E(z)) is the enzymatically active component of the Polycomb Repressive Complex 2 (PRC2) and trimethylates Lysine 27 in Histone H3. The genome wide profiles of this histone mark ($H3K27me_3$) correlate with the transcriptionally repressed states of Polycomb target genes. Recently we developed a system to genetically manipulate canonical histones genes in Drosophila and showed that $H3K27me_3$ mediates the repressive function of PRC2 in wing imaginal discs. Screening for additional Polycomb dependent histone modifications we identified the phosphorylation of Threonine 11 in Histone H3 (H3T11ph) to be dependent on E(z) activity but not on the activity of a canonical PRC2 complex or on H3K27 trimethylation. Chip-seq experiments showed that H3T11ph marks sites of insulator elements that flank domains of $H3K27me_3$ modified chromatin. Although the kinase that phosphorylates H3T11 is still elusive, or data on H3T11A mutant cells in genetic mosaics indicate that H3T11 phosphorylation

counteracts PRC2 dependent H3K27 trimethylation, suggesting a potential negative feedback loop in Polycomb dependent gene regulation via the activity of E(z).

134 Defining the Role of Heterochromatin and Insulator Partner Protein 1 (HIPP1) in Chromatin Insulator Function and Genome Replication *Emily C. Stow*[1], Ran An[1], Todd A. Schoborg[1], Artyom A. Alekseyenko[2,3], Mariano Labrador[1]. 1) Department of Biochemistry & Cell and Mollecular Biology, The University of Tennessee, Knoxville, TN; 2) Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 3) Department of Genetics, Harvard Medical School, Boston, MA.

Nuclear organization is essential for the regulation of gene expression and genome replication timing. This organization is partly mediated by the binding of insulator proteins to insulator sites throughout the genome. Chromatin insulators contribute to the three-dimensional organization of the genome by stabilizing interactions between distant genomic sites along the chromatin fiber. Classic properties of chromatin insulators include their ability to define chromatin boundaries by preventing the spread of heterochromatin and by blocking enhancer-promoter interactions when located between enhancers and promoters. Insulators contribute to transcriptional regulation by selectively allowing contacts between enhancers and regulatory regions of genes. Recent studies show that insulator proteins and DNA replication-related factors bind similar genomic regions, and that the *Drosophila* insulator protein Suppressor of Hairy Wing [Su(Hw)] co-immunoprecipitates with proteins involved in establishing replication origins. These studies link insulators with DNA replication, but details about the precise mechanism of insulator activity during genome replication remain unknown. In this study, we analyze the relationship between Su(Hw) and Heterochromatin and Insulator Partner Protein 1 (HIPP1), a newly identified insulator protein. We use transgenic flies and fluorescence microscopy to better understand the relationship between Su(Hw) and HIPP1 and their activity during genome replication. Our observations strengthen the notion that there is a functional connection between insulator proteins and genome replication, and suggest a mechanism by which HIPP1 facilitates the release of genomic contacts mediated by Su(Hw) during DNA replication. Additionally, we have used CRISPR Cas-9 technology to generate mutations in the HIPP1 gene. Our results show that HIPP1 inhibits Su(Hw) enhancer-blocking activity, and impedes cell proliferation when driven for expression with an inducible Gal-4 promoter. We also observe significant differences in replication timing and the chromosomal localization of replication-associated factors in wild type, overexpressing, and mutant HIPP1 cells.

135 En route to a signature of *trans* inter-homolog pairing in haplotype-resolved genomes *Jelena Erceg*[1], Jumana AlHaj Abed[1], Anton Goloborodko[2], Bryan R. Lajoie[3], Geoffrey Fudenberg[2], Nezar Abdennur[2], Maxim Imakaev[2], Ruth B McCole[1], Son C. Nguyen[1], Eric F. Joyce[1], Tharanga N. Senaratne[1], Mohammed A. Hannan[1], Guy Nir[1], Job Dekker[3], Leonid A. Mirny[2,4], Ting (C.-ting) Wu[1]. 1) Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; 2) Department of Physics, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA; 3) Howard Hughes Medical Institute and Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605-0103, USA; 4) Institute for Medical Engineering and Science, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA.

Nuclear organization is established in part through chromosomal interactions in three-dimensional space, including abundant *cis* contacts as well as *trans* contacts. Although *trans* interactions are implicated in gene regulation, development, and cancer translocations, they are nevertheless much understudied in a genome-wide manner due to their low abundance. Furthermore, the complexity of *trans* interactions is increased in diploid organisms, which have two nearly identical copies of each chromosome, and thus may involve interactions between the homologous maternal and paternal chromosomes.

Drosophila is an ideal system for studying *trans* interactions, as homolog pairing occurs in interphase somatic cells from early embryogenesis to adulthood. Here, we crossed two sequenced strains of *Drosophila* and then generated comprehensive haplotype-resolved Hi-C maps from embryos at a stage when homolog pairing is initiated. Using stringent filtering, we identified a potential Hi-C signature for *trans* inter-homolog interactions. Furthermore, our haplotype-resolved Hi-C maps showed highly concordant TADs between homologous chromosomes, with TAD boundaries correlated with transcriptionally active regions. We have also used the hybrid embryos to generate multiple *Drosophila* cell lines, which will serve as a validation of the presumptive Hi-C signature for homolog pairing in embryos. Taken together, this study provides detailed guidance to inspection of *trans* contacts, especially homolog pairing, in haplotype-resolved genomes. We thank the L Aiden and G Cavalli groups for discussion. This work was supported by awards from NIH (EFJ, JD, LAM, C-tW), Harvard Medical School (C-tW), EMBO (JE), and WR Hearst Foundation (RBM).

136 Zelda and GAGA factor likely function to define the chromatin landscape necessary for genome activation *Katharine Schulz*[1], Eliana Bondra[1], Daniel McKay[2], Melissa Harrison[1]. 1) Biomolecular Chemistry, University of Wisconsin Madison, Madison, WI; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

Following fertilization, the zygotic genome remains transcriptionally silent while the developmental program of the embryo is launched by maternally supplied factors. Within hours, the genome is activated and these maternal factors are degraded in a hand-off known as the maternal-to-zygotic transition. The *Drosophila melanogaster* transcription factor Zelda (ZLD) is responsible for the activation of hundreds of genes during this transition. We have previously demonstrated that ZLD is required to maintain chromatin accessibility at a subset of its binding sites, and that this accessibility facilitates binding of additional transcription factors and gene expression. Notably, the genes associated with these ZLD-dependent sites tend to be expressed early in development, prior to wide-spread genome activation. While the regulators that maintain accessibility at the ZLD-independent sites remain unidentified, our analyses identified enriched motifs suggesting that the transcriptional regulator GAGA factor (GAF) may be involved. We propose that ZLD and GAF are both required to remodel the embryonic genome and to activate gene expression. While ZLD activates early gene expression, GAF may be required to initiate wide-spread genome activation slightly later. The ability of both ZLD and GAF to facilitate chromatin accessibility is reminiscent of pioneer factors, a class of specialized transcription factor that remodel chromatin by directly targeting nucleosomal DNA. Thus, studies of pioneer factors provide a framework to better understand how ZLD and GAF mechanistically function. Fluorescence recovery after photobleaching experiments demonstrated that ZLD recovers quickly, suggesting that it does not share the unusually high affinity for nucleosomes that has been observed with pioneer factors. Further, live imaging of fluorescently tagged endogenous ZLD in the early embryo revealed that ZLD does not remain bound to mitotic chromosomes. We suggest that the rapid nuclear divisions in the early embryo may create a permissive chromatin state, negating the need for a canonical pioneering function. Thus, ZLD and GAF may take advantage of a unique chromatin environment to access their binding sites, and we are currently testing this model.

137 Repetitive sequences on the X chromosome guide dosage compensation *Victoria Meller*, Sonal Joshi. Dept Biological Sci, Wayne State Univ, 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. Elegant studies have resolved the mechanisms of local MSL complex spreading and transcriptional elevation, but the extraordinary selectivity of the MSL complex for the X chromosome has never been explained. We previously demonstrated that the siRNA pathway, and siRNA from a family of X-linked 1.688 g/cm^3 satellite repeats (1.688^X repeats), promote X-recognition. This suggested that a siRNA-dependent mechanism could act through cognate sequences to help identify the X chromosome. To test this idea we integrated transgenes containing 1.688^X repeats on an autosome and demonstrated that these increase MSL recruitment and gene expression in surrounding regions. Placement of 1.688^X repeats opposite a lethal autosomal deletion achieves partial rescue of males, demonstrating functional compensation of autosomal chromatin. Females block formation of the MSL complex and are not rescued. The 1.688^X repeats are therefore *cis*-acting elements that guide dosage compensation. Furthermore, 1.688^X siRNA enhances rescue of males with the lethal deletion, but only when 1.688^X DNA is present on the intact homolog. We propose that the siRNA pathway promotes X recognition by enhancing the ability of 1.688^X DNA to attract compensation in *cis*. The dense distribution of 1.688^X sequences along the X chromosome suggests that they play a primary role in determining X identity during dosage compensation.

138 Interaction between co-activators and PRC1 during development: A key transitional state? *Hyuckjoon Kang*[1,2], Youngsook Jung[3], Kyle McElroy[4], Barry Zee[1,2], Heather Wallace[1,2], Peter Park[3], Mitzi Kuroda[1,2]. 1) Genetics and Medicine, Harvard Medical School, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Biomedical Informatics, Harvard Medical School, Boston, MA; 4) Department of Biology, Tufts University, Medford, MA.

Initially discovered in Drosophila, Polycomb group (PcG) proteins comprise chromatin-modifying complexes whose gene silencing activity is not only critical for developmental body patterning but also strongly implicated in diverse diseases in humans. While many PcG proteins and their biochemical properties have been identified, how PcG proteins are targeted to chromatin and the mechanism governing transition between PcG-silenced and active chromatin states remain poorly understood. We purified PRC1 and PRC2 complexes with Pc and E(z) as bait proteins, respectively, using BioTAP-XL, a crosslinking and tandem affinity purification approach. BioTAP-XL was followed by separate DNA (ChIP-seq) and protein (mass spectrometry) analysis from the same chromatin-based pull-down. We found that PRC1 strongly interacts with transcriptional co-activators Fs(1)h and Enok/Br140 during embryogenesis. Fs(1)h is the Drosophila ortholog of BRD4, a bromodomain protein that binds to acetylated histone marks on active chromatin and is a key transcriptional co-activator in mammals. Enok and Br140 are orthologous to subunits of the mammalian MOZ/MORF histone acetyltransferase, which is highly implicated in human leukemias. We found that the interaction between PRC1 and MOZ/MORF is strongest in embryos, and diminished in differentiated cell types, such as S2 cells and larval imaginal discs. Analyses of co-binding regions of Pc and Br140 in

embryos and S2 cells suggest that the PRC1 and MOZ/MORF interaction is highly specific to poised enhancers linked to developmental genes. We propose that this previously unrecognized, strong interaction between PRC1 and transcriptional co-activators directs a critical 'bivalent' regulatory state. Bivalency may help explain the fidelity of transitions in chromatin state directed by transcription factors during development.

139 Modulating *cis-/trans*-promoter competition in *Drosophila* *Jack R. Bateman*, Justine E. Johnson. Dept Biol, Bowdoin College, Brunswick, ME.

In *Drosophila*, pairing of maternal and paternal homologs can permit *trans*-interactions between enhancers on one homolog and promoters on another, an example of a phenomenon called transvection. Although such interactions have been observed at many loci in the Drosophila genome, the parameters that govern enhancer action in *trans* remain poorly understood. We recently described a transgenic approach to better understand enhancer action in *trans* in *Drosophila melanogaster*, where homologous chromosomes are paired stably together throughout interphase. Using fluorescent reporters, we showed that the eye-specific enhancer *GMR* is capable of acting on two concurrent promoter targets, one in *cis* and one in *trans*, and that the two promoters compete for the enhancers's activity, with the enhancer in *cis* being strongly preferred. We hypothesized that the strong activation of the *cis*-promoter and relatively weak activation of the *trans*-promoter reflects that the strength of activation is a function of the relative enhancer-promoter distance; put simply, the closer a promoter is to the enhancer, the more activity it will receive when competing with another promoter. To test this hypothesis, we asked whether altering the relative distances between the enhancer and the two promoters could skew the enhancer's preference for one promoter over the other. We find that increasing the size of the loop that must form between the enhancer and the promoter in *cis* via insertion of lambda DNA leads to a decreased activation of that *cis*-promoter, with a concomitant increased activation of a competing promoter in *trans*. Furthermore, we used loss-of-function mutations in *CapH2*, which encodes a component of the Condensin II complex that antagonizes pairing of homologous chromosomes, to increase the degree of pairing and therefore bring the *trans*-promoter into closer proximity to the enhancer. As predicted, this resulted in an increased activation of the *trans*-promoter by *GMR*, with a concomitant decrease in the activation of the competing promoter in *cis*. Our data suggest that an enhancer's preference for a promoter in *cis* is at least party due to its physical proximity relative to a promoter in *trans*.

140 Argonaute2 cooperates with LaminB to repress transcription at Lamin-associated domains in *Drosophila melanogaster* *Ezequiel Nazer*[1], Madoka Chinen[1], Ryan Dale[2], Elissa Lei[1]. 1) Nuclear Organization and Gene Expression Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Argonaute proteins are commonly known as core components of RNA silencing pathways. However, Argonaute proteins have also been shown to possess nuclear functions, such as regulation of transcription, splicing and chromatin architecture. Previous work showed that *Drosophila* AGO2 functions directly on euchromatin to promote enhancer-promoter interaction at the homeotic *Abd-B* locus independently of the RNA interference (RNAi) pathway. ChIP-seq analysis revealed that AGO2 binds thousands of sites in the genome, raising the possibility that AGO2 could modulate global chromatin architecture. To identify factors that can function with AGO2 to regulate transcription, we performed immunoaffinity purification of AGO2 from nuclear extracts followed by mass spec analysis. Interestingly, we found that LaminB is enriched among the top AGO2-associated proteins. Reciprocal co-immunoprecitation validated the specificity of this interaction, and biochemical fractionation assays confirmed that both proteins reside in chromatin and nuclear matrix fractions. To directly assess the global role of both proteins in transcription, we performed nascent RNA-Seq upon depletion of either AGO2 or LaminB in Kc167 cells. We found that both proteins co-repressed a highly significant number of genes, particularly those located at the borders of Lamin-associated domains (LADs). In order to assess the physiological role of AGO2 in transcriptional regulation, we performed mRNA-Seq in null versus RNA slicing catalytic activity mutant female larvae. Strikingly, we observed de-repressed transcription of spermatogenesis genes in the absence of AGO2, independent of its catalytic activity. One of the de-repressed genes is *nht*, which encodes a key upstream activator of spermatogenesis gene expression. Null mutation of *nht* suppresses the up-regulation of spermatogenesis genes observed in *AGO2* null mutants, suggesting that AGO2 acts upstream of *nht* to silence the spermatogenesis gene program. Given that *nht* is located within a LAD harboring flanking AGO2 chromatin binding sites, we hypothesized that AGO2 and LaminB could modulate chromatin topology to repress *nht*. Circular chromosome conformation capture assays (4C-Seq) using the *nht* promoter as bait showed a decrease in the frequency of interactions within the LAD upon AGO2 or LaminB knockdown. We conclude that both proteins may repress transcription at LAD borders by regulating chromatin architecture.

141 Why there are no crossovers on chromosome 4 (and how to make them) T. Hatkevich[2], K. Kohl[3], S. McMahan[1], M. Hartmann[2], A. Williams[3], *J. Sekelsky*[1,2]. 1) Department of Biology, University of North Carolina, Chapel Hill, NC; 2) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 3)

Department of Biology, Winthrop University, Rock Hill, SC.
In most sexually reproducing organisms, crossover formation between homologous chromosomes is necessary for proper chromosome disjunction during meiosis I. During meiotic recombination, a subset of programmed DNA double-strand breaks (DSBs) are repaired as crossovers, with the remainder becoming noncrossovers (often with gene conversion). Whether a repair intermediate is designated to become a crossover is a highly-regulated decision that integrates several crossover patterning processes. These include interference (discovered in *Drosophila* more than 100 years ago), the centromere effect (discovered in *Drosophila* more than 80 years ago) and crossover assurance (discovered in other insects more than 80 years ago). Together, these patterning processes produce a non-random crossover distribution. In *D. melanogaster* females, this means that crossover density is highest on in middle of each major arm and low near the ends, particular the centromere end. Crossovers are completely absent on chromosome 4, but it is unknown whether this is due to crossover patterning, sequence, chromatin, or other effects.
Because the mechanisms that generate crossover patterning have remained elusive, it has been difficult to assess the relationship between crossover patterning and meiotic chromosome behavior. We show here that meiotic crossover patterning is lost in *Drosophila melanogaster* mutants that lack the Bloom syndrome helicase (Blm). In *Blm* mutants, interference and the centromere effect are lost, resulting in crossovers being distributed more uniformly along chromosomes. Crossovers even occur on chromosome *4*, suggesting that the lack of crossovers on this chromosome in wild-type females is a consequence of active crossover patterning processes.
Regulated distribution of crossovers between chromosome pairs (crossover assurance) is also lost in *Blm* mutants. This results in an elevated frequency of homologs that do not receive a crossover, which in turn leads to elevated nondisjunction.

142 Novel role of nuclear periphery and nuclear ‘shuttle’ proteins in heterochromatic double strand break repair. *Taehyun Ryu*, Carla D’Agostino, Xiao Li, Quinn Cowan, Irene Chiolo. Molecular and Computational Biology, University of Southern California, Los Angeles, CA.
Proper repair of double-strand breaks (DSBs) in heterochromatin is essential for preventing aberrant recombination and maintain genome integrity. In Drosophila cells, ‘safe’ homologous recombination (HR) repair of heterochromatic breaks relies on a specialized pathway that relocalizes damaged sequences away from the heterochromatin domain before strand invasion[1]. We discovered that heterochromatic DSBs relocalize to the nuclear periphery to continue HR repair[2]. Relocalization depends on nuclear pore and inner nuclear membrane proteins (INMPs) that anchor repair sites to the nuclear periphery via Smc5/6-interacting proteins of the STUbL/RENi families[1,2] . Anchoring is specifically mediated by the Nup107-160 nuclear pore sub-complex and INMPs Koi and Spag4[1]. Both the initial block to HR progression inside the heterochromatin domain, and the targeting of repair sites to the nuclear periphery, rely on SUMOylation and the SUMO E3 ligase[2,3]. I will discuss our most recent discoveries on this pathway, including a network of SUMO-E3 ligases participating in this regulation[3] and the surprising function of nuclear pore 'shuttle' proteins in relocalization. We characterize the importance of the nucleoplasmic role of the nuclear pore proteins with separation function studies. Relocalization defects lead to massive aberrant recombination in heterochromatin, radiation sensitivity, chromosome rearrangements and formation of heterochromatic micronuclei[2,3], suggesting the importance of this pathway in preventing genome instability and tumorigenesis. Our studies so far reveal a critical role for SUMOylation in the spatial and temporal regulation of HR repair in heterochromatin, and identify the nuclear periphery as a specialized site for heterochromatin repair in a multicellular eukaryote.
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143 Switching Injury Response: Polyploidy Associated Hypertrophy as An Alternative to Compensatory Proliferation *Erez Cohen*[1], Don Fox[1,2]. 1) Department of Cell Biology, Duke University School of Medicine, Durham, NC; 2) Department of Pharmacology & Cancer Biology , Duke University School of Medicine, Durham, NC.
In numerous organs, tissue injury is repaired through compensatory proliferation, a regenerative injury response which leads to restoration of tissue morphology and mass. However, numerous tissues have been shown to respond to tissue injury by a distinctly different, non-proliferative process known as hypertrophy. Following injury, hypertrophic tissues increase the size and DNA copy number (ploidy) of remaining cells to restore lost organ mass. While hypertrophy is clearly important to repairing numerous injured organs such as the mammalian liver, this repair process does not always restore tissue morphology and function. Thus, understanding molecular differences between hypertrophy and the more regenerative compensatory proliferation process could improve efforts to regenerate

organs. To approach this question, Using genetic ablation, we established that the *Drosophila* pylorus undergoes compensatory proliferation when injured during juvenile (larval) stages, and then switches to a hypertrophic repair program when injury occurs during adulthood. Studying this process, we can now molecularly interrogate the regulation underlying the decision to undergo hypertrophic or proliferative injury responses. We will discuss recent work identifying the time frame in which the switch between proliferation and hypertrophy occurs. This study provides valuable new insights into conserved mechanisms of organ injury repair using a novel model system.

144 Mitotic gene expression dictates the mechanism of tissue repair in *Drosophila* *Janelle Grendler*, Vicki Losick. MDI Biological Laboratory, Bar Harbor , ME.

A key step in tissue repair and regeneration is to replace cells that have been lost or damaged by injury. One strategy occurs by restoring cell number through cell proliferation and another occurs by increasing cell size through polyploidization, known as wound-induced polyploidization (WIP). Previously we found that polyploid cells arise in the adult fly epithelium in response to injury and are required for tissue repair. During WIP the extent of polyploidy is tuned to precisely restore tissue mass and maintain organ size. Therefore, the degree of polyploidy and the mechanisms that produce polyploid cells must be tightly regulated. One of the key regulators of polyploidization, we identified, is Yorkie (Yki). Yki is the conserved co-transcriptional activator of Hippo signaling pathway, known to regulate cell proliferation and survival. Yet, in the adult fly epithelium Yki is required for entry into the endocycle and the transcriptional program driving cells to polyploidize and not proliferate remains unknown. Here we found that known Yki cell growth and cell cycle transcriptional targets: *bantam, myc, e2f1, and cycE* are induced during WIP and their expression is dependent on Yki. Myc, E2F1, and CycE are required for endoreplication, where as microRNA, *bantam*, appears to be dispensable. In the absence of Yki, ectopic expression of *cycE* is sufficient for the fly epithelial cells to endoreplicate post injury. We hypothesize that in our fly wound healing model the lack of cell division is based on the same principle seen during developmentally programmed polyploidy. Yki is required to stimulate entry into S phase, but the cells fail to express necessary mitotic machinery to enter M phase, as consequence cells endoreplicate instead of undergoing mitotic division during wound repair. Consistent with this hypothesis, the adult fly epithelial cells constitutively express Frizzy-related APC ubiquitin ligase (APC/C^{Fzr}), which is known to target the mitotic cyclins, Cyclins B and B3, for proteolytic degradation. In conclusion, the mechanism of wound repair, cell proliferation or polyploidization, appears to be determined by the cell's competence to express mitotic cyclins.

145 Delineating the Mechanism of Compensatory Cellular Hypertrophy in Follicular Epithelium *S. Row*[1], Y. Tamori[2], PK. Lo[3], R. Perez[4], WM Deng[1]. 1) Department of Biological Science, Florida State University, Tallahassee, Florida; 2) National Institute of Genetics, Mishima, Japan; 3) University of Maryland, School of Medicine, Baltimore, Maryland; 4) Florida State University, College of Medicine, Tallahassee, Florida.

In multicellular organisms, cell competition assists in maintaining tissue homeostasis by eliminating cells exhibiting lower fitness states. In endocycling *Drosophila* follicle cells, generating a mosaic of *mahj* mutants results in their elimination (apoptosis) by post-mitotic cell competition (PCC). As a consequence, there is a non-autonomous homeostatic response to this tissue damage, termed 'Compensatory Cellular Hypertrophy' (CCH). In CCH, seemingly random wild-type cells in the mosaic increase in volume by enhanced Insulin signaling and accelerated endocycling, to make up for the local loss of tissue volume by PCC. Most epithelial cells exist in a state of balanced cellular prestress in a tissue, and evidence from our studies indicate that PCC potentially alters this cellular prestress in cells. When this balance is lost in 'random' cells, they respond via mechanotransducers to become hypertrophic. To explore whether mechanotransducers are *required* for CCH, we screened mechanotransduction related cellular components by knocking them down (using RNAi lines) in wild-type follicle cells of the PCC system discussed above. From the *in vivo* screen, we identified NompC - a TRP channel that functions as a spring-gated ion-channel - as a key player in eliciting CCH. This data suggests that the change in the inherent tension of the tissue as a result of PCC is registered by NompC channels in the compensating cells, which then recruit sub-cellular mechanisms to induce a higher endocycling rate via Insulin signaling, resulting in CCH. This study provides crucial insight into the delicate but tightly regulated balance between intercellular communication, growth regulation, and tissue mechanobiology in epithelial tissue.

146 Large genetic screen identifies FGF signaling in the trachea as a regulator of body size in *Drosophila* *Anne Færch Jørgensen*[1,2], Daniel Krüger Smith[1], E. Thomas Danielsen[1], Sine Kragh Petersen[1], Dylan F. M. Marple[1], Stanislav Nagy[1], Kim Rewitz[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) R&D, Novo Nordisk A/S, Måløv, Denmark.

Reaching an appropriate body size is crucial for the metabolic rate, lifespan, ecological and social fitness of an organism. However, what determines final body size remains elusive. To identify factors that control growth and body

size, we screened the secretome and the plasma membrane receptome for regulators of body size.
In *Drosophila* growth is largely restricted to the larval stage and the pupae size reflects the final size of the adult. Therefore, we conducted an RNAi screen for alterations in pupal size, to identify regulators of final body size.

Remarkably, knockdown of the FGF receptor *breathless* (*btl*) caused the most dramatic size change of all genes. This receptor is predominantly expressed in the trachea and is essential for tracheal development. Knock down of *btl* in the trachea reduces the growth rate and critical weight, which causes a decrease in final size. Animals with reduced expression of *btl* in the trachea also have reduced expression of *Drosophila insulin-like peptide 3* (*Dilp3*) and show accumulation of Dilp2 in the insulin producing neurons, indicating a role for insulin. Our results indicate that FGF signaling in the trachea may be the major regulator of body size in *Drosophila* through regulation of systemic insulin signaling.

147 The Mechanome of Asymmetric Cell Division *Tri T Pham*[1,2], Jonne Helenius[3], Daniel J. Müller[3], Clemens Cabernard[1]. 1) Biology, University of Washington, Seattle, WA; 2) Biozentrum, University of Basel, Basel, Switzerland; 3) D-BSSE, ETH Zürich, Basel, Switzerland.

Asymmetric cell division (ACD) generates cellular diversity and is an important process during development. Stem cells in particular utilize ACD in order to self-renew the stem cell yet generate differentiating siblings. Some stem cells undergo both physical and molecular ACD and it is unknown how biophysical parameters, such as cortical tension, stiffness or osmotic pressure generate physical asymmetry. We use *Drosophila* neural stem cells (neuroblasts) to study the contribution of biophysical parameters on ACD. We are combining fluorescence microscopy with atomic force microscopy (AFM) to measure the dynamics of the actomyosin network and cortical stiffness of cultured neuroblasts. Our measurements indicate that cortical stiffness gradually increases during metaphase before it suddenly drops at early anaphase and then quickly increases to a maximum value at mid anaphase. Interestingly, we detect high stiffness values on the apical cortex at anaphase although Myosin is barely detectable in this region. Since our results suggest that cortical stiffness does not necessarily correlate with Myosin levels, we then use Particle image velocimetry (PIV) and FRET biosensors to measure both cytoplasmic streaming and active cortical tension during ACD. Our FRET data shows that the apical cortex is compressed at early metaphase and is being stretched from anaphase onward. The combination of these measurements allow us to propose a model, explaining how changes in physical parameters contribute to the establishment of sibling cell size differences during mitosis.

148 Interactions between Cell Division and Epithelial Cell Polarity *Gayaanan Jeyanathan*, Ulrich Tepass. Cell and Systems Biology, University of Toronto, 25 Harbord Street, Toronto, ON.

Cell division is essential for proper development of a unicellular zygote into a multicellular organism. However, cell division challenges tissue architecture as cells in division change shape and completely reorganize their cytoskeleton. We are interested in the question of how cell division relates to epithelial cell polarity and the maintenance of epithelial integrity. Recent studies have begun to explore the need for a number of polarity proteins such as Dlg, Lgl, Scribble and Cdc42 for spindle orientation in symmetrically dividing epithelial cells. On the other hand, how the polarity machinery response to the dramatic changes in cell organization that occur during division is not well understood.

In a genetic screen for novel epithelial polarity regulators, we have identified a gene required for mitotic exit. Analyzing this mutant, we found that dividing cells show a marked reduction in the surface abundance of polarity proteins such as Crumbs (Crb). Furthermore, we observed that polarity regulators such as Crb and Cdc42 show genetic interactions with the cell cycle regulator String (Stg)/Cdc25. The loss of cell division seen in *stg* mutants rescues polarity defects. Conversely, overexpression of Stg, which increases cell division, worsens polarity defects. These data show that cell division profoundly challenges the effectiveness of the epithelial polarity machinery to maintain epithelial architecture.

The loss of polarity proteins is a known driver of epithelial-mesenchymal transition (EMT) and tumour progression. To test whether enhanced proliferation drives tumour progression beyond overproliferation, we accelerated the cell cycle in wing imaginal discs. We found that enhanced proliferation causes cells to lose epithelial polarity and tissue integrity, and to exhibit traits characteristic of EMT such as high MMP1 expression, increased F-actin, and loss of cell-cell contacts. We are now testing whether accelerated cell cycle progression in combination with a compromised polarity machinery supercharges tumor progression. In summary, our findings suggest that cell division exposes epithelial tissues to morphogenetic stress that sensitizes epithelia to polarity defects and enhances tumour progression beyond overproliferation.

149 Defective glial phagocytosis results in cell corpse accumulation and age-dependent neurodegeneration *Johnny Elguero*[1], Jon Iker Etchegaray[2], Jenn Tran[1], Mel Feany[3], Kim McCall[1]. 1) Boston University, Boston, MA; 2) University of California San Francisco, San Francisco, CA; 3) Harvard Medical School, Boston, MA.

Throughout development and to maintain tissue homeostasis, cells die and must be cleared. Failure in the

clearance of cell corpses is associated with disease states such as auto-immunity. In the brain, glia are the main phagocytes, and as such are responsible for the clearance of neuronal corpses and debris which may arise from developmental death as well as disease or injury. Several lines of evidence suggest that glial phagocytosis plays a crucial role in neurological diseases including Alzheimer's disease; however, the mechanisms underlying this role remain unknown. In Drosophila melanogaster, we have found that the glial phagocytosis receptor Draper (MEGF10/JEDI-1 in mammals) is required for the maintenance of brain tissue homeostasis. Specifically, the absence of Draper in glia results in an accumulation of neuronal cell corpses and progressive neurodegeneration. While the presence of uncleared cell corpses is the logical result of a defect in clearance, the mechanisms underlying the neurodegeneration observed in *draper* mutants remain unknown. In other tissues, persistent cell corpses can lead to an inflammatory response. We are therefore currently using qPCR as well as genetic and pharmacological approaches to explore the role of inflammation in *draper*-associated neurodegeneration.

150 Dysfunctional VMAT potentiates selective loss of dopaminergic neurons in *parkin* mutant flies Antonio Tito[1,3], Yi-Ting Liu[4], Sonal Nagarkar-Jaiswal[5,6], Danna Ghafir[7], Shebna Cheema[8,9], Mian Jiang[8], Hugo Bellen[5,6], Jay Hirsh[4], *Sheng Zhang*[1,2,3]. 1) Center for Metabolic and Degenerative Diseases, Brown Foundation Institute of Molecular Medicine (IMM), McGovern Medical School, University of Texas Health Science Center at Houston (UTHealth), Houston, TX; 2) Department of Neurobiology and Anatomy, McGovern Medical School, UTHealth, Houston, TX; 3) Neuroscience and Human Genetics Programs, Graduate School of Biomedical Sciences (GSBS), the University of Texas Health Science Center at Houston (UTHealth), Houston, TX; 4) Department of Biology, University of Virginia School of Medicine, Charlottesville, VA;; 5) Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX;; 6) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 7) Wiess School of Natural Sciences, Rice University, Houston, TX; 8) Department of Natural Sciences, University of Houston-Downtown, Houston, TX; 9) Integrative Molecular and Biomedical Sciences Program, Graduate School of Biomedical Sciences (GSBS), Baylor College of Medicine, Houston, TX;.

Parkinson's disease (PD) is caused by the selective loss of the dopaminergic (DA) neurons in the substantia nigra region of brain. One important question in PD study is why these DA neurons are particularly vulnerable, although most of the identified PD genes such as Parkin are largely widely expressed. Notably, dopamine itself is a chemically labile and can become oxidized to toxic byproducts while induce the accumulation of harmful molecules. Accordingly, dopamine toxicity has long been suspected to play a role in selective neuronal loss in PD. Vesicular monoamine transporter (VMAT) is essential for proper vesicular storage of dopamine and its regulated release. Increasing evidence have linked VMAT dysfunction with PD. Here, we examined the sole VMAT homolog (dVMAT) in *Drosophila* and its role in potentiating the selective vulnerability of DA neurons in sensitized *parkin* mutant background. We show that dVMAT overexpression can promote dopamine release, while depletion of dVMAT causes severe loss of total dopamine levels but a buildup of cytosolic dopamine in fly brain. Furthermore, removal of both dVMAT and *parkin* lead to a selective loss of DA neurons primarily in the protocerebral anterior medial clusters of the brain, supporting the potential involvement of cytoplasmic dopamine in the selective destruction of DA neurons. Our results further reveal the intrinsic differences in the dynamics of intracellular dopamine handling among different DA neuron clusters in the brain, and suggest a potential role of this difference in the regional specificity of neuronal loss in PD.

151 Identification of a Neural Modulator of Locomotor Activity That Can Compensate for Loss of Dopamine *Karol Cichewicz*, Ryan Sangston, Olga Olejniczak, Jacqueline Parker, Jay Hirsh. Biology, University of Virginia, Charlottesville, VA.

Parkinson's disease (PD) is the second most common neurodegenerative disease that manifests by death of dopaminergic (DA) neurons in the substantia nigra pars compacta, that causes severe locomotor impairments. It has been known for decades that 30-80% of DA neurons die before symptoms appear (Hornykiewicz 1966, Cheng et al. 2010), leading to the likelihood that compensatory mechanisms play a role in maintaining normal brain function as the dopaminergic system degenerates. Here we identify a gene with such compensatory activities. Genetic and pharmacological models of *Drosophila* neural DA-deficiency phenocopy locomotor symptoms of PD (Riemensperger et al. 2011, Cichewicz et al. 2016). We conducted a selection/breeding experiment in our DA-deficient background, in which we established two subpopulations differing in locomotor activity. The low activity population phenocopies pharmacological DA-deficiency (Cichewicz et al. 2016), whereas the high activity population shows normal levels of locomotor activity, yet is still fully lacking brain DA. We genetically mapped loci contributing to the high activity phenotype using a genome wide association study (GWAS), combined with whole genome sequencing and brain RNA-Seq. GWAS identified several regions on the X and the 2nd chromosome, and the RNA-Seq indicated a single X chromosome gene showing high differential expression, located within one of the GWAS peaks. This gene, CG32581, is a putative ubiquitin ligase that is deleted in the low activity DA-deficient flies, but present in the high activity population. CG32581 is adjacent to CG8974, with the two genes organized as direct repeat, sharing 95%

identity. Manipulation in expression of these genes results in expected behavioral effects. RNF5 is a human ortholog of CG32581 and CG8974 that negatively regulates mitochondrial fission (Zhang, 2012). Its activity is regulated by DJ-1/PARK7 (Parkinson protein 7), a neuroprotective protein mutated in some cases of familial PD. We are thus examining influences of our fly genes on mitochondrial physiology, and the potential role of mitochondrial function in brain circuits modulating locomotor activity.

152 Tip60 HAT/HDAC2 balance promotes neural health in an Alzheimer's disease *Drosophila* model *Priyalakshmi Panikker*, Felice Elefant. Department of Biology, Drexel University, Philadelphia, PA.

Appropriate histone acetylation homeostasis is critical for neural health and function and is maintained by the antagonistic activity of histone acetlytransferase (HAT) and histone deacetylases (HDAC). Disruption of this fine tuned epigenetic balance causes significant cognitive deficits that are a debilitating hallmark of neurodegenerative disorders, including Alzheimer’s disease (AD). Insight into HAT based mechanisms underlying cognition and neuropathology still remains unclear. We generated a robust GAL4 responsive Tip60;APP *Drosophila* model system that enables us to modulate Tip60 HAT levels in neural circuits of choice under AD neurodegenerative conditions, *in vivo.* Its use led to our exciting discovery that Tip60 is critical for cognitive processes and remarkably, protects multiple cognitive neural circuits impaired in the brain during early AD associated neurodegenerative progression. Our findings support a model by which Tip60 promotes neuroprotection by epigenetically reprogramming gene sets that together protect and/or promote cognitive function. To test this model, we performed gene expression analysis on 15 cognition associated fly genes with well- characterized human homologs. qPCR was performed on cDNA isolated from staged 3rd instar larval brain tissue expressing either APP alone (APP) or with additional Tip60 (Tip60^{WT}; APP). Remarkably, we found that while expression of all of the 15 cognition genes tested was repressed in the APP larval brain, expression of 13 of these genes was either partially or fully restored in the Tip60^{WT};APP larval brain. We next tested possible epigenetic mechanisms for such Tip60 transcriptional rescue under APP neurodegenerative conditions. Thus, we selected 5 of the Tip60 rescued cognition genes and assessed levels of Tip60, HDAC2 and histone acetylation enrichment at these loci using ChIP analysis. Remarkably, we found that Tip60 binding affinity is reduced at all 5 gene loci in APP larval heads and restored by increasing Tip60 levels in the Tip60^{WT};APP larval heads. Conversely, HDAC2 binding is significantly enhanced at some of these same Tip60 cognitive genes in the APP larval heads and substantially reduced by increasing Tip60 levels. Accordingly, Ac levels at each of the H4K5,12,and 16 marks at Tip60 cognition gene loci are significantly reduced in the APP brain and restored by increasing Tip60 HAT levels in the brain. Together, our results support a model by which increasing Tip60 in the APP neurodegenerative fly brain restores Tip60 HAT binding and acetylation levels at cognition genes by displacing inappropriate HDAC2 binding, thus activating Tip60 cognition genes to promote neural health.

153 A rapid autophagy response is induced by axon injury and may mediate the signal transduction to axon degeneration Haiqiong Wang, Xu Cao, Zhao Wang, Qingyao Wang, *Yanshan Fang*. IRCBC, SIOC, Chinese Academy of Sciences, Shanghai, Shanghai, China.

Axon degeneration is a prominent feature of spinal cord injury and many human degenerative diseases. Studies of the Wallerian degeneration slow (Wld^{S}) mouse indicate that axon degeneration is an active process, however, the underlying mechanisms remain elusive. To understand the self-destruction program of axon degeneration, we have developed a novel model of nerve injury using the *Drosophila* wing. The fly wing is translucent, allowing us to highlight the axons using fluorescent proteins and to monitor axonal changes in response to traumatic injury and aging in *live* flies.

In this study, we expressed mCherry-labeled Atg8a (an homologue of mammalian LC3) in the wing nerve to visualize and investigate autophagy *in vivo* during aging and upon acute injury. Formation of Atg8a/LC3 puncta is widely used as an autophagosome marker (Klionsky et al., 2016). During normal aging, we found that the basal levels of axonal autophagy (evident by the formation of mCherry-Atg8a puncta) are low in general, even in aged flies. However, upon axotomy, there is a rapid and massive autophagy induction in the distal segment of the injured axons. The response can be seen as early as 30 minutes after injury and persists for days until the axons start to degenerate.

The injury-induced autophagy requires the core ATG genes, as RNAi-knockdown of *Atg1*, *Atg12* or *Atg17* almost completely abolishes this response. Interestingly, no spontaneous axon degeneration is observed with knockdown of the above ATG genes. This data indicates that maintenance of axonal integrity during normal aging does not require axonal autophagy, however, it may play a critical role when axons are injured or stressed such as under disease conditions.

Further investigation reveals that, although overexpression of Wld^{S} or Nmnat can block many early injury responses such as Ca^{2+} influx, MAPK pathway activation and mitochondrial malfunction and protect injured axons from degeneration, they cannot block the injury-induced axonal autophagy. This result suggests that the rapid induction of

axonal autophagy may be an additional pathway that is activated upon axon injury and transduces the injury signal to the action of axon degeneration. Indeed, in a targeted screen for autophagy genes involved in axon degeneration, we identified an autophagy-related gene whose downregulation significantly delays injury-induced axon degeneration. The ongoing experiments are to understand the molecular mechanisms of this gene and axonal autophagy in transuding injury signals to axon degeneration.

154 Seizure and ataxia-linked mutations in a Golgi t-SNARE cause synaptic retraction, frequency-dependent hyperexcitability and reduced dendritic growth in *Drosophila*. R. Praschberger[1], S. Lowe[2], N. Malintan[1], N. Patel[1], H. Houlden[1], D. Kullmann[1], S. Krishnakumar[1,3], M. Usowicz[2], J. Hodge[2], J. Rothman[1,3], *J. Jepson*[1]. 1) Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom; 2) School of Physiology and Pharmacology, University of Bristol, Bristol, UK; 3) Department of Cell Biology, Yale School of Medicine, New Haven, United States.

Mutations in the Golgi t-SNARE *GOSR2* cause progressive myoclonus epilepsy (PME), a devastating neurological syndrome associated with epilepsy, ataxia, myoclonus, motorneuron denervation and early mortality (Corbett et al. (2011) *Am J Hum Genet*). GOSR2 is required for ER-Golgi transport during the secretory pathway - a fundamental process common to all cells. Thus, the basis for such a selective neurological impact of *GOSR2* mutations is unclear. We have investigated this issue using novel *Drosophila* models. The *Drosophila* genome contains a single *GOSR2* orthologue, *membrin*, loss of which results in lethality prior to the L2 larval stage. We generated wild type and PME-mutant *membrin* transgenes and globally expressed these in a *membrin* null background to generate *GOSR2*-PME models. Expression of PME mutant *membrin* rescued early lethality of *membrin* null flies, but resulted in reduced L3 larval locomotion and subsequent pharate-adult lethality. These results suggest that PME-linked *membrin* mutations are hypomorphic. Previous work has demonstrated a critical role for the secretory pathway in regulating dendritic, but not axonal, growth (Ye et al., (2007) *Cell*). Consistent with this, we found that growth of larval ddaC sensory-neuron dendrites, but not axons, was severely perturbed by *membrin* mutations. More surprisingly, using the larval neuromuscular junction as a model synapse, we uncovered a previously unappreciated role for Membrin in regulating trans-synaptic stability and motorneuron excitability. We observed extensive synaptic retraction at *membrin* mutant synapses, accompanied by cytoskeletal fragmentation and reduced expression of trans-synaptic cell adhesion molecules. Paradoxically, *membrin* mutant synapses also exhibit frequency-dependent hyperexcitability. We hypothesise that similar cellular phenotypes may underlie ataxia, motorneuron denervation and epileptic/myoclonic seizures in *GOSR2*-PME patients. Furthermore, we speculate that neurons may be uniquely vulnerable to reduced Golgi trafficking due to their extensive dendritic membrane volume and rapid changes in protein transport and secretion required during synapse remodelling. Our *Drosophila*models now provide a platform to uncover critical cargos transported via the secretory pathway that contribute to these complex neurodevelopmental and physiological alterations.

155 Dystroglycan, a non-integrin ECM receptor is required for selective permeability barrier in the brain *Andriy Yatsenko*, Halyna Shcherbata. MPRG Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Dystroglycan (Dg) is a conserved critical ECM receptor responsible for processes relevant to total organismal health. It is a key component of the dystrophin glycoprotein complex (DGC), which connects the Extracellular Matrix (ECM) to the actin cytoskeleton via the cytoplasmic protein Dystrophin (Dys). Dg has distinct functions from Dys, and apart from its role in muscle maintenance, it is involved in energy homeostasis, photoreceptor differentiation and neuromuscular junction establishment. In humans, defects associated with Dg are called dystroglycanopathies, which are characterized by severe brain abnormalities. Recently, we also reported that the precise levels of Dg in the nervous system are critical, as lower levels slow down neuronal stem cell division, while higher levels accelerate proliferation and perturb neuron differentiation. As the result, deregulation of Dg in the nervous system leads to the formation of cobblestone-like structures that outgrow the normal contour of the ECM defined brain space. This phenotype is similar to the brain cortex abnormalities associated with cobblestone lissencephaly type II in humans, implying that *Drosophila Dg* mutants can serve as a model for this type of dystroglycanopathy. In addition, we have identified that Dg is expressed in glial cells, and deregulation of Dg in glia is lethal. Glial cells are required for many different functions, one of which is the formation of the blood-brain barrier (BBB) in vertebrates or selective permeability barrier (SPB) in flies, which protects the brain from toxic or unwanted moieties. We show that Dg is expressed in glial cells composing the SPB, and in *Dg* mutant flies the integrity of the barrier is disrupted, resulting in the passing of high molecular weight molecules into the brain. Since Dg is conserved in flies and vertebrates, investigating its role in glia will help to understand better the mechanism of dystroglycanopathies in humans.

156 A *Drosophila* functional characterization of CNV genes that confer risk of schizophrenia. *Gianna W. Maurer*, Stanislav Nagy, Julie L. Hentze, Morten Rose, David Mouritzen, Kim F. Rewitz. Section for Cell and Neurobiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark.

Copy number variants (CNVs) associated with the loss of a limited number of genes are emerging as an important genetic cause of mental disorders. The 1q21.1 CNV is a deletion of up to eight genes that confers specific risk of schizophrenia. Although the genes affected by the CNV have been identified, those that contribute to the pathology of the disorder are unknown. However, the CNV genes offer a promising starting point for deciphering the underlying disease mechanisms in animal models. To characterize the biological impact on brain function of individual CNV genes we used the fruit fly *Drosophila* as a model. We therefore analyzed the consequences of knockdown of homologs of 1q21 CNV genes in the nervous system of *Drosophila*. Knockdown of *alicorn* (*alc*) encoding the β subunit of the AMPK complex, a highly conserved energy sensor, caused sleep fragmentation, susceptibility to stress induced by sleep deprivation and impaired learning. Knockdown of *alc* in the nervous system caused fragmentation of sleep similar to flies with increased dopamine (DA) signaling. Furthermore, we found that *alc* interacts with the DArgic system, suggesting that loss of *alc* causes alterations in DA signaling, which has been linked to the 1q21 deletion syndrome. Our data show that *alc* is expressed in the central complex of the brain and indicate that it is required for sleep homeostasis. Taken together, our results indicate that *alc*, the *Drosophila* homolog of the human *PRKAB2*, could be contributing to the increased risk of developing schizophrenia associated with the 1q21.1 deletion.

157 Implications of Active Genetics *Ethan Bier*, Valentino Gantz, Shannon Xu. Dept Biol, 0349, Univ California, San Diego, La Jolla, CA.

Classic rules of Mendelian inheritance impose several significant constraints on genetic manipulation of organisms (e.g., random segregation of distant loci and coinheritance of closely linked loci). In his talk Dr. Bier will discuss how these “passive” rules of inheritance can be superseded by a new form of “active genetics” based on a self-propagating configuration of CRISPR/Cas9 components. In fruit flies, active genetic elements can act on the opposing chromosome in both somatic and germline cells resulting in their inheritance by nearly all progeny, a phenomenon often referred to as gene-drive. Similar results seen also in mosquitoes and yeast open the door to a new era of genetics wherein the laws of traditional Mendelian inheritance can be bypassed for a broad variety of purposes. One important application of gene-drive technology is the ability to spread anti-parasite effector genes efficiently throughout mosquito populations that carry malaria. Dr. Bier considers the implications of this fundamentally new form of “active genetics”, its applications for gene-drives, potential uses of reversal drives, how split-drive strategies could accelerate genetic manipulations in new and existing model systems, and ethical/Biosafety considerations associated with such active genetic elements.

158 An update from the Genome Disruption Project (GDP): MiMICs, CRiMICS, and human cDNA transgenics *Hugo J Bellen*[1,2,6], Pei-Tseng Lee[1], Sonal Nagarkar-Jaiswal[1,6], Karen L. Schulze[1,6], Yuchun He[1,6], Wen-Wen Lin[1], Hongling Pan[1,6], Hillary Graves[1], Oguz Kanca[1], Sathiya Manivannan[1], Shinya Yamamoto[1], Michael F. Wangler[1], Kenneth Wan[5], Soo Park[5], William Fisher[5], Jonathan Zirin[3], Rong Tao[3], Claire Hu[3], Robert W. Levis[4], Allan C. Spradling[4,6], Stephanie Mohr[4], Kenneth L. Scott[1], Susan E. Celniker[5], Nobert Perrimon[3,6]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 3) Harvard Medical School, Boston, MA; 4) Department of Embryology, Carnegie Institution for Science, Baltimore, MD; 5) Lawrence Berkeley National Laboratory, Berkeley, CA; 6) Howard Hughes Medical Institute.

The Gene Disruption Project (GDP) strives to provide publicly available strains that facilitate access to the *Drosophila* genome and its regulatory and coding elements. Our goal is to tag about 4,000-5,000 genes with MiMIC and CRiMIC. MiMIC is a *Minos* based transposable element (TE) that allows the use of recombination-based tools to manipulate genes *in vivo,* and about 2,000 genes can be tagged using this approach based on the available strains in the BDSC. CRiMIC is an insertional strategy based on CRISPR/CAS9 that allows tagging of genes that do not have MiMIC insertions (~2,000-3,000). These technologies allow tagging of genes with GFP and creation of gene specific GAL4 drivers, enabling numerous elegant applications, some of which will be illustrated. So far about 1,200 genes are tagged with GFP or converted into GAL4 drivers. We also embarked on creating a library of 9,000 transgenic stocks that each contain a single UAS-human cDNA. Currently we are in the process of cloning full length human cDNAs into UAS vectors and about 1,000 transgenes should be available by the summer of 2017.

159 Re-purposing Existing Transgenic Reagents By Genomic HACKing Chun-Chieh Lin[1,2], Darya Task[1], Elizabeth Marr[1], Yi-Ting Chang[1], Margaret Ho[3], Ting Xie[3], Mark Wu[3], *Christopher Potter*[1]. 1) The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY; 3) Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD.

The *Drosophila* community has generated thousand of valuable *GAL4*-based transgenic reagents. These *GAL4* lines have been characterized extensively, and many serve as gold-standards for specific tissue expression (*e.g.*, *repo-GAL4* labels glia, *elav-GAL4* labels neurons, *OK107-GAL4* labels mushroom body neurons).

We aimed to develop a genetic technique that would allow any *GAL4* line to be easily and cheaply re-purposed; for example, via conversion to a QF2/split-GAL4/GAL80-expressing line. Accomplishing this goal requires converting the *GAL4* target sequence to the desired donor gene sequence. Gene conversions occur when genomic double strand DNA breaks (DSBs) results in unidirectional transfer of genetic material from a homologous template sequence. Exogenous or mutated sequence can be introduced through this homology directed repair (HDR). We leveraged HDR and gene conversion to develop a method for *in vivo* genomic editing of existing transgenic insertions. **Homology Assisted CRISPR Knockin** (**HACK**) utilizes the CRISPR/Cas9 system to induce DSBs in a *GAL4* transgene, which is repaired by a single genomic transgenic construct containing *GAL4* homologous sequence flanking a *T2A-QF2* cassette. With two crosses, this technique converts existing *GAL4* lines, including enhancer traps, into functional *QF2* expressing lines. We used HACK to convert the most commonly used *GAL4* lines (labeling tissues such as neuronal, fat, glia, muscle, hemocytes) to *QF2* lines. We also identified 'hot' and 'cold' spots of HDR in the genome. The technique is robust and readily adaptable for targeting and replacement of other genomic sequences. We have further generated donor HACK lines for conversion of *GAL4* to *split-GAL4* reagents (AD and DBD), and to *GAL80*. As an additional example of HACK's utility, we adapted the HACK technique to the purpose of generating a targeted Knockin at a genomic locus that had proven challenging to target via existing Knockin techniques.

160 Transgenic gRNA libraries for tissue-specific CRISPR/Cas9 knock-out screening in *Drosophila* *Fillip Port*[1,2], Jana Frei[1], Katharina Kern[1], Claudia Strein[1], Mona Stricker[1], Lukas Voos[1], Simon Bullock[2], Michael Boutros[1]. 1) German Cancer Research Center, Heidelberg, Germany; 2) MRC-Laboratory of Molecular Biology, Cambridge, UK.

Genetic screens are powerful tools for the functional annotation of genomes. In the context of multicellular organisms, interrogating gene function can be greatly facilitated by methods that allow spatial and temporal control of gene abrogation. Here, we describe a system that allows efficient CRISPR-based disruption of specific target genes in a constitutive or conditional manner. The system is based on liberation of multiple gRNAs from an RNA polymerase II or III precursor transcript by processing of flanking tRNAs. Expression of multiple gRNAs targeting the same gene can bypass limitations arising from in-frame indel mutations or gRNAs with low activity. Moreover, multiplexing of gRNAs that target different loci allows generation of complex genotypes in a single step. Because the tRNA system allows tissue-specific expression of gRNAs, it dramatically decreases the frequency of mutagenesis outside the target tissue that is observed with ubiquitously expressed U6-gRNAs and tissue-specific Cas9. We are currently generating large-scale libraries of *UAS-tRNA-gRNA2x* transgenic lines for systematic, tissue-specific CRISPR/Cas9 knock-out screening in *Drosophila*. We will present the current status of this project and the first functional characterization of these lines.

161 What's new in FlyBase (in its 25th year) *Steven Marygold*, The FlyBase Consortium. FlyBase, University of Cambridge, Cambridge, UK.

Since 1992, FlyBase (flybase.org) has provided an essential online database for the Drosophila research community. In addition to being an integrated repository of genetic, genomic and functional data, FlyBase includes numerous tools to view, query, analyse and download these data. Moreover, it also serves as a hub from which users can navigate to related external resources.

Here, we will present an overview of features and tools recently added to FlyBase, with the aim of promoting their utility to researchers. Novel report pages have been generated for Drosophila models of human disease, functionally related gene groups, and large datasets. Orthology data have been enhanced through the incorporation of the DIOPT orthology set and the provision of a new query tool. Gene reports have been augmented with short, hand-written summaries ('snapshots') of gene function, as well as new graphical summaries ('ribbons') of functional, phenotypic and expression data. Protein domains are now represented graphically within our genome browser, as well as on gene and polypeptide reports. JBrowse has been deployed as an updated genome browser, offering several advantages over the GBrowse instance. The underlying website architecture has also been overhauled, resulting in several enhancements including new faceted searches and improved display of the website on mobile devices. Several new community initiatives have served to enhance interactions between researchers and FlyBase, including the 'FlyBase Community Advisory Group' and a new series of video tutorials. Finally, a new web portal ('Gene2Function') is under development that will facilitate querying of data from different model organisms, with a particular focus on their application to human genetics and disease.

162 Metabolomic Studies in *Drosophila* *Jason Tennessen*, Hongde Li. Department of Biology, Indiana University, Bloomington, IN.

The *Drosophila* metabolome consists of any small-molecule metabolite (<1500 Da) that is found within biological samples. Recent advances in metabolomic technologies allow for the simultaneous measurement of hundreds of these metabolites, which when coupled with the genetic resources available to the fly community, provides an

unprecedented opportunity to study metabolic regulation. No single metabolomic technology, however, is capable of surveying the entire metabolome; therefore, the ideal metabolomic study must be carefully designed to examine the most relevant metabolites. Here we will describe a general strategy for conducting metabolomic studies in the fly. We will introduce the concept of the metabolome, explain why the metabolome varies in a context dependent manner, and provide a general survey of technologies that are available for both the novice and advanced user. Particular attention will be given to the basics of experimental design, the advantages and limitation of current instrumentation, and the differences between steady-state measurements and metabolic flux analysis. We will also provide a general introduction to data analysis and interpretation. Finally, we will highlight recent efforts to generate resources for the fly community and provide key examples of how metabolomic approaches can lead to the discovery of novel metabolic mechanisms.

163 Advances in monitoring calcium dynamics using genetically-encoded sensors in *Drosophila* *Yi Sun*, Hod Dana, Eric Schreiter, Loren Looger, Karel Svoboda, Vivek Jayaraman, Douglas Kim. HHMI/Janelia Research Campus, Ashburn, VA.

Calcium is a ubiquitous signaling molecule in various tissues and is implicated in many biological processes. Monitoring intracellular calcium changes has thus provided critical insights into a variety of biological processes. As part of the Janelia GENIE (Genetically-Encoded Neuronal Indicators and Effectors) project, we are optimizing genetically-encoded calcium indicators (GECIs) for neuroscience applications, although the tools we have developed have found wide application in other disciplines where calcium sensing is required.

Calcium has been linked with electrophysiological events in neurons and is considered as a proxy for neural activity, but protein sensors of calcium activity have historically been plagued by limitations in sensitivity and kinetics. We recently developed the GCaMP6 family of ultrasensitive green fluorescent GECIs (Nature, 2013) that have been widely used in several genetic model organisms including *Drosophila*. We tested these GECIs by using one-photon imaging to evaluate responses to electrical stimulation in the larval neuromuscular junction (NMJ) and two-photon imaging to examine odor responses in the antennal lobe, and demonstrated that GCaMP6 GECIs provide significant improvements over previous generations of green GECIs. GCaMP6 indicators have since been successfully used in combination with cell-type-specific drivers to record the activity of complete population of neurons in the fly brain at high spatial and temporal resolution. They have also enabled the activity of different compartments of the same neuron to be monitored simultaneously.

We are now extending the range of calcium detection in both the spectral and temporal domain. Firstly, we made the calcium integrator, CaMPARI (Science, 2015), which we used to reveal brain-wide multi-synaptic pathways activated by stimulating fly olfactory sensory neurons using odors and optogenetics. Secondly, we made red-shifted calcium sensors, such as jRGECO1 and jRCaMP1 (eLife, 2016). These red calcium sensors, when combined with green calcium sensors, enable simultaneous dual-color imaging for studying transformations of representations across synapses in a pathway. Finally, we are generating calcium affinity variants for imaging various types of neuronal cells with different calcium dynamics. I will describe these tools and discuss how they have enabled dramatic progress in studies of neural circuit function in *Drosophila*.

164 Whole-animal functional and developmental imaging with multi-view light-sheet microscopy *William C Lemon*, Phillip J Keller. Janelia Research Campus, Howard Hughes Medical Ins, Ashburn, VA.

Comprehensive in-vivo imaging of central nervous system (CNS) development in the Drosophila embryo presents a number of challenges. Imaging rapid cellular dynamics in large specimens over long developmental periods requires high spatiotemporal resolution in all dimensions as well as good physical coverage and minimal photo-toxicity. We have developed a light-sheet microscopy platform that provides all these characteristics and is providing new insights into the development and function of the Drosophila nervous system in all developmental stages. Our multi-view light-sheet microscope technology is capable of volumetric imaging speeds of at least 5 Hz, providing a comprehensive functional assay of the Drosophila CNS, while maintaining full developmental competency over days at a time. To further extend these capabilities, we recently developed isotropic multi-view (IsoView) light-sheet microscopy, which allows for the extremely rapid imaging of very large specimens via simultaneous light-sheet illumination and fluorescence detection along four orthogonal directions. Here we present an introduction to state-of-the-art light-sheet microscopy approaches to live imaging of Drosophila development and function and demonstrate how the unique capabilities of this technology can specifically advance our understanding of the development and function of the CNS. We include key considerations in the design of light-sheet microscopy experiments, sample preparation, fluorescent marker strategies, experiment automation, image processing, and data extraction as well as an outlook toward future technology developments.

165 Recombinase-facilitated Fine-mapping of Neural Circuits using Split Cre Haojiang Luan, Matthew Roberts, Fengqiu Diao, Feici Diao, *Benjamin White*. Lab Molecular Biol, NIMH, Bethesda, MD.

The Gal4-UAS system has been the workhorse for cell-type-specific manipulation in *Drosophila*, but in many cases

the expression patterns of Gal4 drivers are not sufficiently specific to permit selective manipulation of individual cell types. This problem is particularly acute in studies of the nervous system, where the diversity of cell types is huge, and single neurons (or bilateral pairs of neurons) often have unique functions. Numerous combinatorial methods for delimiting Gal4 activity to small groups of neurons have been developed, but reproducibly targeting cellular manipulations to identified neurons in the fly brain remains a challenge.

To facilitate efforts at fine-mapping neuronal function in the fly, we have developed a system which uses a Split Cre recombinase to parse the expression pattern of a Gal4 driver into subsets of neurons based on lineage. Our technique relies on the neuroblast-specific excision of an otherwise ubiquitously expressed floxed Gal80 cassette and on the use of split inteins to effect the reconstitution of Split Cre fragments within individual lineages. An initial parsing of a Gal4 expression pattern is achieved using a two-fragment Split Cre molecule, which can be further divided into three fragments to additionally refine the Gal4 pattern. We show how such iterative parsing can be applied to identify single neurons required for specific behaviors.

166A The Role of CG31345 in Regulating Microtubule Polymerzation *Safiyah H Alzahrani*, Susan A Spencer. Biology, Saint Louis University, Saint Louis, MO.

Microtubules are filaments that are involved in cell structure, intracellular transport, and cell division. Microtubule polymerization and depolymerization in cells are carefully regulated. We have been investigating the role of a small Ca^{2+} binding protein, known as CG31345, in regulating tubulin polymerization into microtubules. Nothing is known about CG31345's function. Nonetheless, it is similar to human calcyphosine (CAPS), human calcyphosine like (CAPSL) and to the Drosophila protein CG10126. The alignment between CG31345 and CG10126 shows that the proteins are 65% identical and 85% similar. Work in our lab suggests that CG10126 helps to regulate mitosis. Consequently, we hypothesize that CG31345 may also be a microtubule-binding protein that regulates tubulin polymerization. We have used a variety of approaches to investigate this hypothesis and have found the following: 1) co-immuneprecipitation experiments indicate that CG31345 binds tubulin specifically in the presence of Ca^{2+} 2) immunohistochemistry indicates that CG31345 co-localizes with tubulin in the cytoplasm, and 3) tubulin polymerization assays indicate that CG31345 seems to inhibit microtubule polymerization. The last finding indicates that CG31345 acts in opposition to CG10126, which has been found to promote microtubule polymerization. From these results, we propose that CG31345 is a tubulin-associated protein that influences microtubule regulation in vivo.

167B Identification of gene interactions with the tyrosine kinase Abl during cell migration *Alexandra Byrd*, Traci Stevens. Biology, Randolph-Macon College, Ashland, VA.

Cell migration is an essential process in which cells of multicellular organisms move during development to form tissues and organs. The actin cytoskeleton, which underlies the cell membrane, determines the shape of cells, and regulated rearrangements of this actin network drive cell migration. Abl, a non-receptor tyrosine kinase, acts as a key regulator of the actin cytoskeleton, thereby regulating cell shape and motility. A mutant version of Abl, Bcr-Abl, has increased tyrosine kinase activity relative to Abl and is linked to leukemia in humans. In cell culture, expression of Bcr-Abl has dramatic effects on actin structure and cell motility. The exact cellular pathways by which Abl and the mutant Bcr-Abl regulate cell migration are not completely known. Our laboratory uses the embryonic epithelium of *Drosophila* as a model system to study Abl pathways that regulate cell migration during development. *Drosophila* embryos expressing the mutant Bcr-Abl in the epithelium die, with defects in processes requiring regulated cell migration including dorsal closure and head involution. Previous studies in our laboratory have identified several genetic modifiers of these activated Abl-dependent defects. In order to verify a role for these interacting genes in the context of normal Abl signaling pathways that regulate development, mutant alleles of genes previously found to modify Bcr-Abl-dependent phenotypes were combined with overexpression of wild-type Abl in the embryonic epithelium, which causes embryonic lethality with defects in head involution. As controls, we found that mutant alleles of *shg* and *ena*, genes previously found to interact with wild-type Abl in the embryonic epithelium, genetically modified phenotypes associated with overexpression of Abl in this system. Mutant alleles of several other genes that genetically interacted with Bcr-Abl expression also modified defects associated with overexpression of wild-type Abl, with eight suppressing and three enhancing defects associated with Abl overexpression. Taken together, these studies provide new insights into components the Abl signaling pathways that direct cell migration *in vivo.*

168C Characterization of a novel actin regulator, HtsRC *Julianne Gerdes*[1], Andrew Hudson[1], Katelynn Mannix[1], Lynn Cooley[1,2,3]. 1) Genetics, Yale University, New Haven, CT; 2) Cell Biology, Yale University, New Haven, CT; 3) Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

Development of eggs and sperm occurs in clusters of sister cells that are formed through incomplete cytokinesis. These cells remain connected by intercellular bridges called ring canals (RCs), which replace the cytokinetic cleavage furrows. In the *Drosophila melanogaster* female germline, RCs acquire a robust actin cytoskeleton that supports the dramatic increase in RC lumen diameter over the course of egg chamber development. The Kelch-

Cullin3 RING ubiquitin ligase functions at RCs to regulate filamentous actin. Genetic studies show that the *hu-li tai shao (hts)* gene is also involved in the accumulation of F-actin to RCs, and a product of this gene, HtsRC, is a substrate of the Kelch-Cullin3 RING ubiquitin ligase. In this study, we demonstrate that HtsRC, produced by a germline-specific *hts* transcript, is necessary for F-actin accumulation. Recently generated mutations in the HtsRC coding region block accumulation of F-actin to RCs and cause a dramatic reduction in RC size. Strikingly, homozygous mutant females are not sterile but have severely reduced fecundity. Ectopic expression of HtsRC in the somatic follicle cells results in accumulation of filamentous actin aggregates that contain several markers of RCs. These results support the conclusion that HtsRC protein is necessary and sufficient for recruiting F-actin assemblies. To gain insight into the mechanism by which HtsRC regulates actin, we are using *in vitro* assays that test for the binding, bundling, and *de novo* polymerization of actin filaments. The predicted HtsRC protein is conserved only in the Drosophila family and contains no known functional domains. To identify functionally important domains, we have generated transgenic HtsRC fragments to test their ability to localize to RCs and generate ectopic actin aggregates. Studying HtsRC as both a novel protein and as a substrate of a well-characterized E3 ubiquitin ligase will enhance our understanding of existing actin regulatory mechanisms, and may provide new insights into the regulation of F-actin structures.

169A Molecular guillotine: a novel approach to inhibit kinesin motor activity *Zhiyi Lv*[1], Jan Rosenbaum[2], Takuma Kanesaki[1], Timo Aspelmeier[2], Joerg Grosshans[1]. 1) Institute for Developmental Biochemistry, Medical school, University of Goettingen, Goettingen, Niedersachsen, Germany; 2) Institute for Mathematical Stochastics, University of Goettingen, Goettingen, Niedersachsen, Germany.

Motor proteins are important for transport and force generation in a variety of cellular processes and morphogenesis. Genetic analysis is hampered by the essential function of many motor proteins, however. For example, since Kinesin-5 serves indispensable functions during mitosis, it is difficult to genetically investigate its role in processes and cytoskeletal organization during interphase. Here we designed a conditional mutant of Kinesin-5, inserting a protease cleavage site between the head domain and the stalk. Coexpression of the protease leads to specific cleavage and loss of Kinesin-5 function. Employing this “molecular guillotine”, we have investigated the function of Kinesin-5 for the dynamics of the cytoskeleton in syncytial *Drosophila* embryos. We confirmed the previously reported mitotic function in spindle morphogenesis and chromosome segregation. We found a novel interphase function of Kinesin-5 in suppressing cytoskeletal fluctuations. Embryos injected with the protease displayed much higher second-scale fluctuations of centrosomes than controls. These together with other data from our laboratory support a model that the syncytial cytoskeletal network is stabilized by overlapping microtubules originating from adjacent asters. These data exclude a model that Kinesin-5 at the overlapping microtubules generate repulsive forces driving centrosome fluctuations. The “molecular guillotine” can be widely applied in other motor proteins with accessible necks due to the conserved structures of kinesin proteins.

170B Spire and Cappuccino Team Up to Establish Body Axes During Oogenesis *Margot Quinlan*[1], Christina Vizcarra[2], Alexander Bradley[1], Haneul Yoo[1], Joseph Walsh[1]. 1) Chemistry and Biochemistry, UCLA, Los Angeles, CA; 2) Department of Chemistry, Barnard College, New York, NY.

Spire and Cappuccino (Spir and Capu) are classical polarity factors that stand out because they are essential for both dorsal/ventral and anterior/posterior axis determination. Genetic and biochemical data indicate that they collaborate to assemble an actin mesh in the oocytes of animals, including Drosophila and mammals. Both Spir and Capu nucleate actin assembly independently in vitro, which leads to the question of why two actin nucleators would be required to build the same structure. We found that a direct interaction is required for Spir and Capu to function in vivo. We took a combined in vitro/in vivo approach to determine the mechanism of the collaboration. In vitro, we observe potent enhancement of actin assembly when Spir is added to Capu in pyrene actin assays containing profilin and capping protein – conditions that block most independent nucleation by Spir. In this assay, Spir could be enhancing Capu’s nucleation, elongation, or both activities. We are now able to biochemically separate Spir’s nucleation and barbed end binding activities with point mutations in individual WH2 domains. In vitro, we find that nucleation by Spir and not barbed end binding is necessary for synergy. We asked whether the same is true in vivo by replacing endogenous Spir with mutant transgenes in Drosophila. To our surprise, neither of Spir’s activities is exclusively required. Flies with Spir that cannot nucleate independently or Spir that cannot bind barbed ends have an actin mesh in their oocytes and are fertile. We interpret this as evidence that either nucleation or barbed end binding by Spir is sufficient in vivo and that Spir enhances both nucleation and elongation by Capu.

171C Filamin actin-binding and dimerization domain fulfill distinct functions in Z-disc cohesion Nicanor González-Morales, *Frieder Schoeck*. Department of Biology, McGill University, 1205 Dr Penfield Avenue, Montreal, Quebec, CANADA H3A 1B1.

Sliding filaments mediate muscle contraction in the sarcomere, the smallest functional contractile unit of muscle. Many proteins contribute to the elastic and contractile properties of muscles, most notably myosin thick filaments,

which are anchored at the M-line, and actin thin filaments, which are anchored at the Z-discs that border the sarcomere.

Here we show how the giant scaffold protein filamin contributes to myofibril assembly. In humans, mutations in filamin-C result in severe myopathies, but the underlying molecular function is not well understood. Filamin has two actin-binding CH domains followed by up to 24 Ig domains, the last of which functions as a dimerization domain. We use the *Drosophila* indirect flight muscle as a model system to study the filamin ortholog Cheerio, which has the same domain organization, only lacking two Ig domains, Ig 7 and 10. We show that *Drosophila* filamin localizes to the Z-disc and identify the filamin domains required for interaction with the titin ortholog Sallimus. In addition, we demonstrate a strong genetic interaction of filamin with titin and actin. Intriguingly, we analyze specific filamin mutations that disrupt either the dimerization and titin-binding domain or the actin-binding domain and that show specific disruptions of the Z-disc. In the absence of the filamin dimerization and titin-binding domain, myofibrils fray, and the Z-disc breaks up laterally. In the absence of the filamin actin-binding domain, thin filaments no longer properly attach with their plus ends at the Z-disc. Our results show that actin-binding and dimerization domain of filamin carry out distinct functions in Z-disc cohesion.

172A Dynamic regulation of the cell polarity protein Crumbs during epithelial morphogenesis *Anna Bajur*[1], Alejandro Vignoni[1,2], Ivo Sbalzarini[1,2], Elisabeth Knust[1]. 1) Max-Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany; 2) Center for Systems Biology Dresden, Pfotenhauerstrasse 108, 01307 Dresden, Germany.

During morphogenesis cells undergo dramatic remodelling and shape changes in order to give rise to specialized tissues and organs. These events involve a profound amount of mechanical stresses that are being exerted on the cells. Therefore, the tight regulation of cell polarity as well as the maintenance of cellular junctions are both indispensable to maintain tissue stability. The Crumbs (Crb) protein complex is one of the key regulators of cell polarity and maintenance of adherens junctions in epithelia of the *Drosophila* embryo. Previous genetic studies have shown that the amount and localization of Crb must be strictly regulated to ensure proper development of epithelial tissues. Despite having plethora of experimental data, there is a huge gap in our knowledge on the regulatory mechanisms involved in Crb distribution at the plasma membrane (PM).

In this study, we aim at understanding how Crb localization at the PM is maintained and regulated in the highly dynamic environment of a developing organism, the *Drosophila* embryo. To this end, we are using Fluorescence Recovery After Photo-bleaching (FRAP) and high-resolution live imaging combined with super-resolution microscopy.

Data we obtained so far suggest a spatio-temporal regulation of Crb mobility throughout the development of the *Drosophila* embryonic epidermis. This could be explained by well-established differences in morphogenetic activities along the anterior-posterior and dorsal-ventral axis of the embryo. Another striking observation is that interactions between Crb and cytoskeletal components seem to play an important role in regulation of Crb mobility.

Future work aims at unravelling the molecular basis that underlies Crb distribution at the PM. This will be crucial to understand the role that Crb plays in maintaining cell polarity and tissue integrity.

173B Scribbled mediates tricellular junction formation *Zohreh Sharifkhodaei*, Mary Gilbert, Vanessa Auld. University of British Columbia, Vancouver, BC, Canada.

The scaffold protein Scribbled has major roles in cell polarity, cell migration and cell proliferation in epithelial cells. In Drosophila melanogaster, the Scribbled polarity complex consists of Scribbled (Scrib), Disc large (Dlg) and Lethal giant larvae (l(2)gl). Scrib, Dlg and Lgl are highly conserved tumor suppressor genes and are critical to establish apicobasal polarity by restricting apical proteins from the basolateral surface in epithelial cells. In the columnar epithelia of the Drosophila imaginal disc, loss of Scrib leads to disruption of epithelial organization, loss of polarity, overproliferation, and disc overgrowth. The leucine-rich repeat (LRR) domain of Scrib is essential to maintain the polarized epithelial monolayer and control proliferation. While the PDZ domains are thought to be required for septate junction (SJ) formation independent of Scrib's role in apical polarity. In Drosophila SJs form permeability barriers to exclude solute flow across tissue barriers. A related junction, the tricellular junction (TCJ) is found at the convergence of three bicellular junctions to block paracellular diffusion and maintain the barrier at the corners of cells. As the role of Scrib in the tricellular junction (TCJ) is still unknown, our goal was to understand how Scrib interacts with the two known TCJ proteins Gliotactin and Bark-beetle (Bark). We found that Scrib is concentrated at the TCJ in both columnar and squamous epithelia of the wing imaginal disc and in close proximity with the TCJ proteins Gliotactin and Bark, using a proximity ligation assay (PLA). Previous work identified a complex at the TCJ between Gliotactin and Dlg, and we found that Bark and Gliotactin along with Dlg are all in close proximity at the TCJ . We found that Lgl(2) is not present at TCJ suggesting the Scrib complex at TCJ is different from the polarity complex. To test the interactions between Scrib and other components of the TCJ we carried out RNAi-mediated knock down experiments. Bark knock down led to the loss of Gliotactin from the TCJ and the spread of Scrib and Dlg basolaterally. To determine which domains of Scrib might be important, we utilized previously characterized Scrib alleles that lack one or more of the PDZ domains. Loss of all four PDZ domains disrupted the normal distribution of

Dlg along with Bark and Gliotactin at the TCJ. However Dlg, Gliotactin and Bark were all correctly localized at the TCJ in Scrib alleles lacking the 3rd and 4th PDZ domains and retaining the 1st and 2nd PDZ domains. Overall our findings suggest that Scribbled forms a unique complex at the TCJ along with Bark, Gliotactin and Dlg, and that the presence of the PDZ 1-2 domains is essential for this interaction.

174C Apnoia, a new Crumbs regulator for proper breathing in flies *Kassiani Skouloudaki*, Elisabeth Knust. Max-Planck Institute of Cell Biology and Genetics, Dresden, Saxony, Germany.

The *Drosophila* tracheal system is a model to study the development and function of tubular organs. Maturation of tracheae has been extensively studied, and results contributed to our understanding of the pathology of human diseases such as asthma, COPD etc. Mechanisms such as tracheal inflation or tracheal growth in response to oxygen still remain elusive. Here we characterized *apnoia* (*apn*), a gene that encodes a transmembrane protein containing a lipoprotein lipid attachment site in its extracellular N terminal domain. Apn protein is highly conserved in insect species, but does not appear to have a true orthologue in vertebrate species. *apn* is expressed zygotically in stage 10 embryos, and Apn protein localizes to the apical plasma membrane of epithelial cells, predominantly in the tracheal system. *apn* mutant animals show defects in tracheal inflation at larval stages. Tracheal tubes in mutant larvae are twisted, although tracheal maturation appears normal during development. We found Apn as a partner of Crumbs in a Y2H screen. In *apn* mutant tracheae Crumbs is mislocalized and trapped in large vesicles within the cell. We are currently studying the connection between Apn and Crumbs at the molecular level and its role at the organ and cellular level.

175A The *Merlin* and *expended* genes are involved in Spiny-legs induced planar cell polarity reversal. *Jun Wu*, Marek Mlodzik. Dept. of Cell, Deve and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

Epithelial cells are not only polarized along apical-basal axis, but also within the plane of the epithelium, known as planar cell polarity (PCP). *frizzled/flamingo* (*fz/fmi*) and *fat/dachsous* (*ft/ds*) core PCP genes are required for the PCP establishment. The *frizzled/flamingo* (*fz/fmi*) group includes *fz, fmi, Van Gogh (Vang), prickle(pk), dishevelled (dsh), diego (dgo)* genes. The *fat/dachsous* group includes *ft, ds, four-jointed (fj) genes*. The *prickle* (*pk*) gene encodes both *prickle* (*pk*) and *spiny-legs* (*sple*) splicing isoforms of transcripts. Functionally, Pk and Sple proteins antagonize each other. *pk* isoform is required for planar cell polarity (PCP) in the wing as *pk* mutant wings display abnormal wing hair polarity (readout of PCP), while *sple* mutants display normal PCP in the wing. Overexpression of Sple caused PCP reversal in the wing, partially similar to PCP phenotypes of *pk* mutants as Sple was overly active due to lack of Pk antagonism to Sple. PCP reversal induced by Sple requires both Fz/Fmi and Ft/Ds group core PCP proteins, as the polarity reversal is blocked in fz^- mutant wings or in wings expressing *ft* or *ds* inhibitory RNA (RNAi). To further examine the mechanism of the polarity reversal, we co-expressed Sple with RNAi against genes encoding proteins known to interact with Ft or Ds protein. We discovered *lowfat (lft), dco (discs overgrown), hpo (hippo), ex (expanded) or Mer (Merlin)* knockdown (via RNAi) blocked Sple induced polarity reversal, suggesting these growth genes were involved in the PCP reversal. *hpo (hippo), ex (expanded) or Mer (Merlin)* knockdown activates Hippo growth control pathway via Yorki activation. However, the blockage of PCP reversal seemed to be independent of Yorki activation as co-expression of active form of Yki and Sple didn't block the PCP reversal. This study suggests that Hpo, Ex and Mer are involved in novel PCP regulation that is independent of Yorki activation.

176B The Role of the Retromer Subunit Vps26 in Vesicular Trafficking During *Drosophila* Oogenesis *Rebecca Starble*, Nancy Pokrywka. Vassar College, Poughkeepsie, NY.

During endocytosis, molecules are internalized by the cell through the invagination of the plasma membrane. Endocytosis is required for proper cell function and for normal development in *Drosophila*. One component of the endocytic pathway is the retromer complex, which recycles transmembrane proteins to other parts of the cell such as the plasma membrane and the trans-Golgi network. Previous studies have shown that mutations to the retromer complex result in developmental defects in *Drosophila*. In humans, retromer dysfunction has been implicated in both Alzheimer's and Parkinson's disease, but little is known about the role of the retromer complex in *Drosophila* oogenesis. In the current project, we examined the role of the retromer protein Vps26 in oogenesis by characterizing the phenotype of *vps26-* germline clones. Immunofluorescence was used to visualize the expression of membrane proteins and vesicular trafficking markers in mutant egg chambers. We find that *vps26-* germline clones exhibit nurse cell membrane degradation, indicated by a decrease in the membrane protein syntaxin. Additionally, the border cells of the *vps26-* germline clones had significantly greater LysoTracker stain than wildtype border cells, indicating an increase in lysosomal degradation. These data suggest there is a defect in signaling between the germline and follicle cells.

177C Effect of the small GTPase, Rab10 on membrane growth and cellularization of the early *Drosophila* embryo. *Elliott Holt*, Blake Riggs. Biology, San Francisco State University, San Francisco, CA.

During cellularization a considerable amount of intracellular membrane is rapidly and dynamically added to the ingressing furrow in order to simultaneously encapsulate the many thousands of nuclei forming the cellular blastoderm. The source of this membrane growth is not well understood but this membrane addition is suggested to occur through one of two routes: exocytosis of vesicles to the apical domain and subsequent transcytosis of this apical membrane to the growing furrow; these processes are mediated by conventional cytokinetic machinery and in part by the activity of the small GTPases, Rab11 and Rab5, respectively. Recently, we have observed the small GTPase, Rab10, localizing to ingressing cellularization furrows as well as syncytial pseudoclevage furrows. In RNAi knock down experiments targeted to Rab10, we observe a disruption of normal pseudocleavage furrow formation. This data suggests that Rab10 may act within the furrow formation pathway and may constitute a novel vesicle trafficking route. Furthermore, we are interested in exploring the relationship between Rab10 and ER tubule growth as it relates to furrow formation within the developing embryo.

178A Dissolution of electron-dense plaques during myoblast fusion *Stefanie Lübke*, Julia Hamp, Andreas Löwer, Corinna Heck, Susanne-Filiz Önel. Developmental Biology, Philipps-Universität Marburg, Marburg, Germany.

Muscle development in *Drosophila* is characterized by the fusion of two different types of cells, the founder cells (FCs) and fusion-competent myoblasts (FCMs). FCMs migrate towards, adhere and fuse to founder cells. During somatic myoblast fusion, the recognition and adhesion of those cells is mediated by members of immunoglobulin (Ig) and Cadherin superfamily. The adhesion proteins are localized in a ring-like structure at the point of the cell –cell contact and transfer the fusion signal by their cytodomains, The interacting Ig proteins form a cell communication structure, called FuRMAS, and indirectly recruit the nucleation-promoting factors Scar and WASp to the site of cell contact. Both proteins activate Arp2/3-dependent F-actin polymerization, which is essential for fusion pore formation. At the ultrastructural level, these events are reflected by the appearance of finger-like protrusions and electron-dense plaques that dissolve prior to fusion.

Kette (a homolog of Nap1 and Hem-2) is a component of the Scar/WAVE regulatory complex and its loss results into severe myoblast fusion defects. In *kette* mutants finger-like protrusions still form but the electron-dense plaques fail to dissolve. Genetic interaction studies suggest that *kette* has two functions during myoblast fusion. First, it is required to dissolve electron-dense plaques to bring the membranes into close proximity for fusion. Second, it coordinates the function of the Arp2/3 activators Scar/WAVE and WASp during fusion pore formation.

To identify genes involved either in electron-dense plaques dissolution or fusion pore formation, we performed a modifier screen in the *Drosophila* eye and will present first identified modifiers.

179B Identification of proteins required for prefusion complex dissolution. *Michaela S. Papendieken*, Julia Hamp, Susanne Filiz Önel. Developmental Biology, Philipps-Universität Marburg, Marburg, Germany.

During *Drosophila* myogenesis two different types of myoblasts the founder cells (fc) and fusion-competent myoblasts (fcm) fuse to form the larval body wall musculature. The recognition and adhesion of myoblasts leads to the formation of a cell-communication structure at the interface of FC and FCMs. Myoblast type specific cell surface receptors trigger the recognition of the actin cytoskeleton and the appearance of electron-dense vesicles (called pre-fusion complex) and plaques. Electron-dense plaques seem to represent cellular junction-like structures that need to be dissolved to bring the membranes into close proximity for membrane merge (Hamp et al., 2016). The dissolution of the plaques involves the function of the Scar complex member Kette. However, Scar complex members also play a role in fusion pore formation.

The role of the pre-fusion complex during myoblast fusion is still unknown. In *blow* mutants electron-dense vesicles accumulate and can be observed more frequently than in wild-type embryos. However, Blow has also implicated in F-actin organisation and fusion pore formation. To elucidate the role of Blow during pre-fusion complex dissolution, we performed a global yeast-2-hybrid screen to identify new interaction partner. Here we present the expression pattern of first potential interacting partners and their preliminary analyses.

180C Roles of catalytic and noncatalytic class II PI3K splice variants in autophagy *Jean-Francois Groulx*[1], Steve Jean[2], Sudha Kumar[1], Amy Kiger[1]. 1) University of California-San Diego, San Diego, CA; 2) Université de Sherbrooke, Sherbrooke, QC.

The phosphoinositide 3-kinases (PI3Ks) comprise a family of three classes (class I, II, III) of lipid kinases. While PI3K classes I and III have known roles in autophagy, roles for PI3K class II (PI3KC2) are unknown. Interestingly, PI3KC2 isoforms in mammals and flies are each predicted to encode for two major splice variants: a full-length catalytic protein, and a noncatalytic short variant. However, the contributions of individual splice variants to any PI3KC2 functions are unexplored. In Drosophila, we generated the first variant-specific PI3KC2 (Pi3K68D) genomic deletion alleles. Using autophagy assays in larval fat body, including indicators of Atg8-marked autophagosome levels and flux and of autolysosome acidification and maturation, we identified opposing autophagy requirements for each variant. Interestingly, the catalytic and noncatalytic PI3KC2 isoforms are required to simultaneously inhibit or

derepress, respectively, the normal levels of both autophagy initiation and autolysosome maturation in Drosophila fat body. TEM analysis revealed that while deletion of the short variant led to a block in degradation of autophagic cargo in immature autolysosomes, deletion of only the full-length variant or simultaneously both catalytic and noncatalytic variants together exhibited enhanced autophagy initiation with efficient autolyososome function even in fed conditions. The high level of autophagy inappropriately induced in fed conditions in the absence of full-length PI3KC2 required the canonical Atg1/Vps34 pathway. Together, these results suggest coordinated functions for the PI3KC2 splice variants, with normal roles for full-length PI3KC2 to repress autophagy in fed conditions and the noncatalytic PI3KC2 variant to release this repression with starvation-induced autophagy. Indeed, we found that both PI3KC2 protein forms can physically interact in fat body, and overexpression of the short, noncatalytic PI3KC2 variant was sufficient to shift localization of the full-length, catalytic PI3KC2 protein. Collectively, ours results indicate opposite functions of PI3KC2 catalytic and non-catalytic variants in autophagy at both early and late steps. Currently, we are testing the specific requirements and regulation of PI3KC2 kinase activity, including contributions to lysosome maturation and reformation and the impacts on mTOR kinase control of autophagy regulation.

181A The Sorting Nexin *Snazarus* regulates autophagosome-lysosome fusion *Steve Jean*, Annie Lauzier. Anatomie et Biologie Cellulaire, Université de Sherbrooke, Sherbrooke, Québec, Canada.

Autophagy, the degradation and recycling of cytosolic components in the lysosome, is essential for cell homeostasis. Autophagy is a membrane-mediated process intimately linked to trafficking events. The molecular mechanisms regulating autophagy induction under stress conditions (i.e. starvation) are well characterized. However, how are membrane trafficking events coordinated to account for the increased autophagy demand during stress is poorly defined. Previous work has identified RAB21 and its guanine exchange factor, MTMR13, as essential endosomal regulators of autophagy. Briefly, MTMR13 GEF activity is induced by starvation, which leads to the transient activation of RAB21. Significantly, this activation promotes RAB21 association with VAMP8, a N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins required for autophagosome/lysosome fusion. This RAB21-VAMP8 interaction reroutes VAMP8 trafficking to endolysosomes where it mediates fusion events. How VAMP8 is sorted by RAB21 to endolysosomes is unknown. The sorting nexin (SNX) protein family represents a potential molecular link between RAB21 and VAMP8, given that SNXs are known to regulate cargo sorting from endosomes. We screened by RNAi, all sorting nexins in the fly fat body and identified *Snx3*, *Snx18*, *Snx21* and *Snazarus* as regulators of autophagy. Significantly, only *Snazarus* was required for VAMP8 trafficking. *Snazarus* loss-of-function inhibited autophagosome/lysosome fusion as observed with the GFP:mCherry:Atg8 autophagic sensor and by Ref(2)P accumulation. Importantly, *Snazarus* depletion did not affect general endosomal function in the fly fat body and we found in S2 cells that *Snazarus* and RAB21 interact biochemically. Finally, the closest human orthologue of *Snazarus*, SNX25 is also required for autophagosome/lysosome fusion. This work identified *Snazarus* as a novel regulator of autophagy that mediates VAMP8 sorting to endolysosomes, potentially through a direct interaction with activated-RAB21. As such, *Snazarus* represents a new coordinator of membrane trafficking that links autophagic demand to intracellular traffic.

182B Cdk5 enhances Basal Autophagy by phosphorylating Acinus *Nilay Nandi*[1], Lauren Tyra[1], Helmut Kramer[1,2]. 1) Dept. of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 2) Dept. of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Cdk5 has complicated roles in neurodegeneration and cancer, variably inhibiting or promoting their progression. From a targeted screen, we found that Cdk5 phosphorylates Acinus (Acn) at serine 437. Knocking down Cdk5, or its required cofactor p35, drastically reduces Acn phosphorylation at serine 437. Acn, primarily a nuclear protein, positively regulates basal autophagy [1]. We find that phosphorylation of serine 437 regulates stability and function of Acn. Physiological relevance for this modification was confirmed by a phospho-mimetic S437D mutation in vivo: stabilized Acn[S437D] enhanced basal autophagy and extended life span in wild type or *p35* mutants. A possible mechanism for Acn activity was suggested by its role as a subunit of the ASAP complex, which binds to spliceosomes and regulates a subset of alternative splicing [2, 3]. To test the hypothesis that Acn stimulates autophagy through its function in alternative splicing we compared the transcriptomes of wild type and *acn* larval fat bodies. However, we could not uncover any support for this hypothesis. Furthermore, Acn^{ASAP}, a mutant that interrupts its binding interface with Sap18 and RNPS1, disrupts its function in alternative splicing as predicted [2,3]. Nevertheless, when expressed at endogenous levels in flies, Acn^{ASAP} enhanced basal autophagy and is primarily cytosolic. Thus, this mutant separates the regulatory functions of Acn in splicing and autophagy. Further support for a splicing-independent, non-nuclear role of Acn came from expression of a myristoylated Acn that also displayed enhanced autophagy.

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183C Zonda is a novel early component of the autophagy pathway Mariana Melani[1], Ayelen Valko[1], Nuria Romero[1], Milton Aguilera[2], Gabor Juhasz[3], Maria Isabel Colombo[2], *Pablo Wappner*[1]. 1) Fundacion Instituto Leloir, Ciudad de Buenos Aires, Argentina; 2) Laboratorio de Biología Celular y Molecular-Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. Mendoza, Argentina; 3) Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary Institute of Genetics, Biological Research Centre, Szeged, Hungary.

Autophagy is an evolutionary conserved process by which eukaryotic cells undergo self-digestion of cytoplasmic components. Here we report that a novel *Drosophila* immunophilin, which we have named Zonda, is critically required for starvation-induced autophagy. We have found that Zonda is required at early stages of the process, specifically for Vps34-mediated phosphatidylinositol 3-phosphate (PI3P) deposition. Zonda displays a cytoplasmic distribution under basal conditions, and soon after starvation nucleates in endoplasmic reticulum-associated *foci* that colocalize with autophagosome formation sites, the omegasomes. Zonda nucleation depends on Atg1, Atg13 and Atg17 but does not require Vps34, Vps15, Atg6 or Atg14. We propose that Zonda is an early component of the autophagy cascade necessary for Vps34-dependent PI3P deposition and omegasome formation.

184A Manipulation of targeted mitochondrial DNA double strand breaks in a *Drosophila* model *A. Spierer*[1], D. Yoon[2], D. Rand[1]. 1) Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Department of Neuroscience, Brown University, Providence, RI.

The 37 genes encoded by the mitochondrial genome (mtDNA) must coordinate with ~1200 nuclear genes to perform the mitochondrion's essential cellular functions, like aerobic respiration, nutrient sensing, and apoptosis. Mitochondria replicate independent of the cell cycle, requiring rigorous coordination between these two genomes to ensure effective organellar function. However, the mitochondrial genome is prone to damage, in part due to its close proximity to the OXPHOS complexes it partially encodes. Reactive oxygen species, a byproduct of aerobic respiration, are a form of oxidative damage capable of inducing mtDNA double strand breaks (mtDSBs). While there are hundreds to thousands of mtDNA copies within a given cell, mitochondrial dysfunction and pathological phenotypes will develop once a threshold of damage is exceeded. The aim of this study was to identify nuclear genes that could counteract mitochondrial dysfunction associated with mtDSB damage. We used a Gal4-UAS model to induce targeted, mtDSBs with a mitochondrial-localizing restriction enzyme (MitoRE). Previously, we demonstrated a knockdown in fertility when a MitoRE was driven constitutively in the germ line, via the nanos driver. We identified a regulator of mitochondrial biogenesis, *Spargel*, as a strong candidate gene for partially rescuing fertility in both males and females expressing the MitoRE. Of the fertile flies coexpressing both the MitoRE and *Spargel*, we observed a slight increase in fecundity compared to controls. This experiment serves as a proof-of-concept for identifying specific nuclear genes that can mitigate mtDNA damage. Future work aims to use the Drosophila Genetics Reference Panel (DGRP) to find many more candidate nuclear genes capable of modifying the mtDSB response.

185B Uncovering the subcellular trafficking routes of secreted molecules within secondary cells *F. Castellanos*, B. Kroeger, C. Wilson. Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom.

The underlying mechanisms that regulate how cells traffic secretory molecules are of fundamental cell biological significance in diverse contexts such as physiology, development and disease. We model subcellular trafficking using a highly secretory cell type, the secondary cells (SCs), which are found within the epithelium of the male accessory glands (AGs) of *D. melanogaster*. Despite their low abundance, SCs play key roles in reproduction, specialising in the production and secretion of an essential fraction of the seminal fluid. SCs contain a number of large subcellular compartments (>3 μm diameter) that are responsible for the secretion of these factors. The compartments can be readily defined by acidity and differential Rab GTPase association. We have previously shown that Rab7 localises to the limiting membranes of large acidic late endosomal multivesicular bodies and lysosomes (MVBLs), while Rab11 is found on the surface of non-acidic secretory compartments. Exosomes, which can be labelled by a GFP tagged form of CD63, constitute an important part of SC secretion, and are classically thought to originate from Rab7 compartments. The secretory Rab11 compartments contain dense core granules, whose contents are also released into the seminal fluid. We aim to show how different types of secreted molecules traffic to and from specific compartments to reach the lumen of the gland or other subcellular destinations. For this purpose, we have used wide-field fluorescence and DIC microscopy to study these compartments in high detail. Using the UAS-Gal4/TubGal80ts system, we have pulse chased a diverse set of florescent fusion proteins through the SC secretory pathways, either individually or in different combinations, to determine their trafficking routes. Time-lapse imaging of pulse-chased cells has also provided a better understanding of how different compartments are formed and matured within SCs. Using markers specific for the dense core granules, we have now characterised different stages of non-acidic compartment maturation. This work suggests a novel role for dynamic intraluminal vesicles inside these compartments in the transfer of proteins to dense cores. We are now testing several other molecules with proposed roles in dense core formation. This study has provided a detailed characterisation of secretory trafficking routes within

SCs and has revealed novel aspects of their subcompartmental biogenesis. Furthermore, it presents evidence for new mechanisms of dense core granule regulation via intraluminal vesicles. These findings are relevant to our understanding of other specialised secretory cells involved in important physiological processes such as the glucose-dependent pancreatic beta cells.

186C Sequential trafficking events target White transporter to pigment granules Dongsheng Chen[1], Jian Xiong[1], Yuqing Mike Xiong[4], Antonio Tito[1,3], Zhihua Chen[1], Hongyue Jiang[5], Ge Jessie Jiang[6], *Sheng Zhang*[1,2,3]. 1) Center for Metabolic and Degenerative Diseases Institute of Molecular Medicine, McGovern Medical School at The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, 77030; 2) Department of Neurobiology and Anatomy, McGovern Medical School at The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, 77030; 3) Programs in Human and Molecular Genetics and Neuroscience, The University of Texas Graduate School of Biomedical Sciences, Houston, TX, 77030; 4) The University of Texas-Austin, Austin, TX; 5) Shanghai Jiao Tong University School of Medicine, Shanghai, China; 6) Shandong University School of Medicine, Jinan, Shandong, China 250012.

White, the first fly mutant identified by Morgan over a century ago, is also the most widely used marker in fly studies. The fly eye color is determined by red and brown pigments storied inside pigment granules, the only known lysosomal-related organelle (LRO) in *Drosophila*, in the pigment cells of adult eyes. *white* encodes an ABCG family half transporter, which is believed to form heterodimers with Brown or Scarlet, two other ABCG transporters, to package red and brown pigments, respectively, into the pigment granules. Despite its long history and importance in fly research, there are few systematic studies on the exact expression pattern of White and very limited information on how White protein is specifically targeted to the pigment granules. Using specific anti-White antibodies and genome-tagged White transgenes, we show that White is glycosylated and its expression starts early during embryogenesis and continues into adulthood. At subcellular level, White is specifically localized on the limiting membrane of two distinctive subpopulations of pigment granules, which we propose are defined by the White partners Brown and Starlet, respectively. Besides *white*, large numbers of eye color mutants exist. Among them are the groups of granule genes known to be involved in regulating the discrete steps of subcellular trafficking of membrane proteins among different organelles. Importantly, in different subgroups of granule mutants, White exhibits distinctive subcellular distribution patterns. Analysis of these phenotypes suggests a sequential subcellular trafficking of White by discrete protein complexes, which together orchestras the faithful deliver of White to pigment granules. Finally, there is an absolute requirement of Brown and Starlet for the stability and proper trafficking of White. Our studies thus lay foundation for detailed dissection of mechanisms controlling White trafficking and help our understanding of the biogenesis of LRO and LRO-related diseases.

187A *ER Stress Delays the Cell Cycle in Drosophila Syncytial Embryos* *R. Kyger*, I. Avellano. SFSU, San Francisco, CA.

Organelle inheritance during cell division is poorly studied as compared to chromosomal inheritance. Additionally, the issue of whether or not “organelle checkpoints” exist, and by checkpoint we mean a mechanism to delay the inheritance of malfunctioning organelles, has barely been addressed. In particular, despite the fact that the basic functions of organelles like the Endoplasmic Reticulum (ER) have been well studied, not much is known about the inheritance of the ER during mitosis or if an ER checkpoint exists. To address this deficit, we chose to investigate the mechanism of ER inheritance during mitosis in *Drosophila* syncytial embryos. We hypothesized that ER stress induction would result in an ER inheritance delay during mitosis in *Drosophila* syncytial embryos. To investigate this, we used a transgenic *Drosophila* line that expresses both the ER marker, PDI-GFP, and the DNA marker, H2Av-RFP. Syncytial embryos from these fly lines were injected with DTT, a drug that breaks disulfide bonds, in order to induce ER stress. Furthermore, injection of DTT into syncytial embryos caused ER sheet expansions and cell cycle arrests. At this time, we believe these cell cycle arrests occurred in either S phase or early prophase and further work will need to be done in order to elucidate the exact cell cycle phase. In conclusion, our preliminary results suggest the existence of a cell cycle checkpoint that activates as a result of the ER malfunctioning from ER stress. Moving forward, we also plan to inject Tm (tunicamycin), a drug that blocks N-glycosylation, and perform an RNAi knockdown of Rtnl1 (reticulon), an ER structural protein, to induce ER stress in syncytial embryos. Moreover, we anticipate that these approaches will also cause ER sheet expansions and cell cycle arrests in syncytial embryos. In the future, we also plan to investigate the mechanism of this ER checkpoint.

188B Fic-mediated AMPylation of the ER chaperone BiP is required to maintain visual neurotransmission *Andrew Moehlman*[1], Amanda Casey[2], Junmei Zhang[2], Kim Orth[2,3], Helmut Krämer[1]. 1) Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX; 2) Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX; 3) HHMI, Dallas, TX.

Vision is important for animals to sense their environment and to enable responses to changing conditions. Therefore visual processing must adapt to a wide range of light conditions. In the fly, photoreceptor neurons sense

light changes and transmit these signals to lamina and medulla neurons. We previously found that the single Drosophila *fic* gene is necessary for proper visual neurotransmission and recycling of histamine, the primary vision neurotransmitter. In electroretinogram (ERG) recordings, *fic* null flies displayed reduced responses to the onset of a light pulse (ON transients). Fic catalyzes AMPylation (the transfer of an adenosine monophosphate), a post-translational protein modification conserved in many species. The first biochemically identified substrate of eukaryotic Fic was BiP (*Hsc70-3*), an abundant and ubiquitous ER-resident chaperone. To test whether AMPylation of BiP contributes to the role of Fic in visual transmission, we generated transgenic flies in which endogenous BiP was replaced by wild-type BiP or AMPylation-resistant BiP. The vision defect previously observed in *fic* null flies was phenocopied by AMPylation-resistant BiP. BiP is involved in protein synthesis and secretion as well as regulating the Unfolded Protein Response, which are functions that may be necessary in vision to adapt to changing light conditions. To test whether AMPylation of BiP is required for light adaption, we exposed flies to 72 hours of constant bright light. Both *fic* mutants and flies with AMPylation-resistant BiP, but not wild-type flies, responded with loss of both ON and OFF transients and a reduction in the sustained negative potential (SNP) of photoreceptors. However, both mutants recovered normal OFF transients and SNPs after 72 hours of recovery, suggesting that the stress-induced changes in the mutants reflect a reversible deregulation of a cellular stress-response. These results demonstrate, for the first time, a physiological role for Fic-mediated AMPylation of BiP in the maintenance of visual neurotransduction.

189C Modeling of axonal endoplasmic reticulum network by spastic paraplegia proteins *Cahir J O'Kane*[1], Belgin Yalçin[1], Lu Zhao[1], Martin Stofanko[1], Niamh C O'Sullivan[1], Zi Han Kang[1], Annika Roost[1], Matthew R Thomas[1], Sophie Zaessinger[1], Olivier Blard[1], Alex L Patto[1], Anood Sohail[1], Megan Oliva[1], Juan José Perez Moreno[1], Valentina Baena[2], Mark Terasaki[2]. 1) Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH, United Kingdom; 2) Cell Biology, UConn Health Centre, 263 Farmington Avenue, Farmington, CT 06030-3505, USA.

Axons contain an endoplasmic reticulum (ER) network that is largely smooth and tubular, thought to be continuous with ER throughout the neuron, and distinct in form and function from rough ER; the mechanisms that form this continuous network in axons are not well understood. Mutations affecting proteins of the reticulon or REEP families, which contain intramembrane hairpin domains that can curve ER membranes, cause an axon degenerative disease, hereditary spastic paraplegia (HSP).

Here, we show that these proteins are required for modeling the axonal ER network in *Drosophila*. Loss of reticulon or REEP proteins can lead to expansion of ER sheets, and to partial loss of ER from distal motor axons. Ultrastructural analysis reveals an extensive ER network in every axon of peripheral nerves, which is reduced in larvae that lack reticulon and REEP proteins, with defects including larger and fewer tubules, and occasional gaps in the ER network, consistent with loss of membrane curvature. Therefore HSP hairpin-containing proteins are required for shaping and continuity of the axonal ER network, suggesting an important role for ER modeling in axon maintenance and function.

We are now screening additional HSP gene products for roles in axonal ER, and developing tools to image axonal ER structure and function in live axons.

190A A deficiency screen for genetic interactors of Jagunal in the Drosophila compound eye Sydney Alvarado, Emily Conrad, Jose Ortega, *Nicole Rodrigues*, Blake Riggs. San Francisco State University, San Francisco, CA.

Cell differentiation is generated by asymmetric cell division during mitosis in which two different daughter cells are produced. Preliminary data shows that ER partitions asymmetrically during a symmetrical cell division just prior to cell fate determination in the early *Drosophila* embryo. The mechanisms in which ER asymmetry dictates cell fate are unknown. Our laboratory data has demonstrated that this asymmetrical division is dependent on the presence of Jagunal (Jagn), an evolutionarily conserved ER transmembrane protein. Currently, there are no genes involved in the Jagn signaling pathway that have been identified. In order to identify Jagn interactors, we ectopically expressed a Jagunal RNA interference (JagnRNAi) line in the *Drosophila* compound eye. We observed a disruption in eye topology, generating a rough eye phenotype in 80% of the progeny. We have performed an enhancer-suppressor deficiency screen covering the right arm of the third chromosome, and we have identified 20 enhancers/suppressors of the JagnRNAi rough eye phenotype. These deficiencies include several factors involved in cell signaling and centrosome maturation and function. Future directions include expanding the screen to include deficiencies covering the left arm of the third chromosome for the identification of Jagn interactors. Identification of these factors will provide insight into the molecular pathway involving Jagn in the asymmetric partitioning of the ER.

191B A Novel Role of VCP in Maintaining the Nuclear Structure and Function of End-Dividing Cells *Ya-Chu Chang*, Tzu-Kang Sang. Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan.

The nuclei of metazoan cells undergo drastic morphological change during cell cycle to accommodate DNA replication and the subsequent cell segregation. But how end-dividing cells regulate their nuclear structure to maintain cellular homeostasis is an issue surprisingly with little insight. Through the examination of post-mitotic photoreceptor cells expressing a dominant-negative mutant form of TER94 (*Drosophila* homolog of VCP), we found those end-dividing cells launched a drastic nuclear expansion. VCP is an AAA (ATPase associated with diverse cellular activities) ATPase that has been reported to participate in various cellular events, including ubiquitin-dependent protein degradation, organelle biogenesis, DNA damage response, and cell cycle regulation. VCP is abundant both in the cytosol and in the nucleus, but its role in the nucleus remains largely unexplored. We found the loss of nuclear VCP activity may be responsible for the observed nuclear expansion phenotype as accumulated ubiquitinated proteins are associated with nuclear TER94 when the ATPase activity is compromised. This result further implicates that TER94 may have a role in nuclear protein quality control, and the deficit of this crucial process could lead to aberrant nuclear expansion. The abnormal nuclear structure induced by dominant-negative mutant TER94 was accompanied with altered histone modification, implicating that the gene expression profile could be affected due to the change in nuclear morphology. A growing number of reports recognized spatial and temporal maintenance of nuclear architecture are critical for genome stability and cell homeostasis. Importantly, the changes in nuclear size and shape have been used as a morphometric readout for cancer prognosis and aging. Understanding VCP-mediated aberrant nuclear expansion may provide mechanistic insights of some neoplastic changes and normal aging process.

192C Nuclear Wash functions in multiple nuclear complexes to affect nuclear morphology and processes. *Jeffrey Verboon*, Bina Sugumar, Susan Parkhurst. Fred Hutchinson Cancer Research Institute, Seattle, WA.

Conventionally, Wiskott Aldrich Syndrome (WAS) proteins are activated by upstream signals, usually from Rho family GTPases, to form new branched actin networks in association with the Arp2/3 complex. The WASH subfamily of WAS proteins has essential cytoplasmic roles in oocyte and embryonic development, cell migrations, and endosome sorting and scission, where it functions as part of (or independent from) a WA**SH R**egulatory **C**omplex (SHRC) in a context-dependent manner. It has become clear that WAS family proteins also have nuclear roles separate from their cytoplasmic activities, with most WAS proteins associated with specific transcriptional machineries. Recently, we showed that in addition to having a role in specific transcriptional contexts, Wash also is found throughout the nucleus and acts to affect morphological features of the nucleus. *Wash* depletion in both cells and salivary gland nuclei causes a range of nuclear phenotypes including a crumpled nucleus, increased DNA accessibility, disrupted chromosome organization, altered heterochromatin, and disruption of nuclear compartment markers such as coilin (cajal bodies) and fibrillarin (nucleolus). While several of these phenotypes are attributable to an interaction with the *Drosophila* B-type lamin, Wash's ubiquitous distribution in the nucleus and diverse nuclear phenotypes led us to suspect that Wash affects nuclear morphology as part of multiple complexes. To elucidate these complexes, we performed tandem affinity purification mass spectrometry to identify novel Wash-interacting proteins in fractionated nuclear extracts, as well as identifying native complexes by blue native (BN-) PAGE. We identified 15 high-confidence, novel nuclear interactors, unexpectedly including SHRC proteins, however, this is likely an under-representation. Additionally, we identified 6-10 Wash-containing complexes in nuclear lysates by BN-PAGE. Importantly, we find that Wash's native complexes overlap with native complexes of specific interactors such that the Lamin-Wash complex is different from the SHRC-Wash complex. Consistent with this, SHRC mutants exhibit only a subset of Wash phenotypes. Thus, nuclear Wash acts as part of multiple, separable complexes to affect a diverse set nuclear properties/events. We are currently investigating the protein components and specific functions of each of these complexes.

193A Stratum, a Homolog of the Human GEF Mss4, Partnered with Rab8 Controls the Basal Restriction of Basement Membrane Proteins in Polarized Epithelial Cells *Olivier Devergne*, Gina Sun, Trudi Schüpbach. Department of Molecular Biology, Princeton University, Princeton, NJ.

The basement membrane, a sheet of extracellular matrix lining the basal side of epithelia, is essential for epithelial cell function and integrity, tissue organization, and organ morphogenesis. Importantly, the disorganization and misregulation of the basement membrane have been associated with carcinomas and tumor metastasis. Despite the critical roles of the basement membrane in normal and abnormal behavior of epithelial cells, the molecular mechanisms ensuring the accurate basal secretion of basement membrane proteins, such as Collagen IV and Perlecan, remain largely unknown. In epithelial cells, a specialized pathway is dedicated to restrict the deposition of basement membrane proteins basally. Using the follicular epithelium of the Drosophila ovary as a model system, we previously found that the guanine nucleotide exchange factor (GEF) Crag and appropriate levels of PI(4,5)P2 are critical in ensuring the polarized distribution of basement membrane proteins. Here we report the identification of a new factor of this pathway, a homolog of the mammalian GEF Mss4/RabIF, that we named Stratum. We showed that Stratum is necessary for the basal deposition of basement membrane proteins in polarized epithelial cells. Using

super resolution microscopy, we showed that the loss of Stratum leads to missecretion of Perlecan and Collagen IV apically, forming aberrant layers in close contact with the plasma membrane. Interestingly, we found that Rab8GTPase acts downstream of Stratum in that process. Additionally, the epithelial distribution of Stratum suggests it is required to restrict Rab8 activity basally and thus direct basement membrane protein-containing vesicles towards the basal side of epithelial cells. Together, our results uncovered the necessity of a new GEF/RabGTPase complex, composed of Stratum and Rab8, in specifically coordinating the basal restriction of basement membrane proteins, a critical process for the establishment and maintenance of epithelial cell polarity.

194B X-ray crystallography and computational molecular dynamics of *Drosophila* striated muscle myosin II isoforms predict a basis for isoform-specific properties *James Caldwell*[1], Dan Mermelstein[2], Ross Walker[2], Girish Melkani[1], Tom Huxford[1], Sanford Bernstein[1]. 1) San Diego State University, 5500 Campanile Drive, San Diego, CA 92182; 2) University of California, San Diego, San Diego Supercomputer Center, 9500 Gilman Drive, MC 0505, La Jolla, CA 92093-0505.

Drosophila melanogaster contains one gene, *Mhc*, encoding all striated muscle myosin II isoforms. To gain insight into how alternative exon selection imparts myosin protein isoform biochemical and biophysical specificity, we employed X-ray crystallography and computational molecular dynamics. His-tagged recombinant proteins encoding an indirect flight muscle myosin isoform (IFI) or an embryonic body wall myosin isoform (EMB) were expressed in and purified from the indirect flight muscles (IFM) of engineered fly lines lacking endogenous IFM myosin. The purified myosins retain ATPase activity similar to that of their corresponding untagged isoforms. Myosin subfragment-1 (S1) containing each myosin heavy chain motor domain and the essential light chain was crystallized and we determined the three-dimensional structure of IFI S1 at 2.5 Å resolution and EMB S1 at 2.2 Å resolution (PDB 4QBD). They are the first insect myosin protein structures determined by X-ray crystallography. The enzymatic state for both structures is post rigor, as determined by comparison with known myosin structures. For EMB, two copies of the myosin molecule with slight conformational differences were resolved in the asymmetric unit. The electron density revealed a citrate molecule (contained in the crystallization condition) in the nucleotide-binding pocket. For IFI, there is one myosin molecule in the asymmetric unit, with ADP in the nucleotide-binding pocket. A 500 nanosecond molecular dynamics simulation was run using the GPU accelerated AMBER 14 software suite and the FF14SB force field on the EMB crystal structure as well as on a model of IFI created by introducing the alternative amino acid sequences into the EMB structure. Analysis of the dynamics data included calculating the root mean square fluctuations. This resulted in the identification of two regions in IFI with significantly higher mobility than EMB: one contained the C-terminal portion of the relay domain and the other was the converter domain. The differences in conformation within regions of the proteins encoded for by alternative exons suggest a source of the observed physiological differences in the embryonic and adult flight muscle fibers. (Funded by NIH R01GM32443 to SIB)

195C Defining the role of mechanotransduction downstream of prostaglandin signaling in regulating border cell migration *Emily F Toombs*, Maureen Lamb, Tina L Tootle. University of Iowa Carver College of Medicine, Iowa City, IA.

Collective cell migration – the coordinated movement of tightly or loosely associated cells – is important for development and tumor invasion. Many signals control collective cell migration including mechanotransduction, or the transfer of physical force into electrical or chemical signals. Mechanotransduction is mediated by the direct connection of the cytoskeleton to the nucleoskeleton via the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. Another mechanism regulating cell migration is prostaglandins (PGs), short-range lipid signals. Our lab has shown that PGs control actin remodeling via regulating specific actin binding proteins. Importantly, actin binding proteins play key roles in mechanotransduction. Despite this, PG signaling has not been previously implicated in mechanotransduction. Here we present the first evidence that PGs regulate the LINC Complex during the collective and invasive cell migration of the border cells during oogenesis. The border cells are a cluster of 6-8 somatic cells that delaminate from the epithelium and migrate between the nurse cells to the oocyte border. We <u>hypothesize</u> that PG signaling modulates border cell migration by regulating perinuclear Fascin to control LINC complex function. This hypothesis is based on our prior studies that revealed PG signaling regulates Fascin, an actin bundling protein widely implicated in cell migration and highly expressed in the border cells. This regulation occurs, in part, by PGs modulating the localization of Fascin, including Fascin's perinuclear localization. Loss of either PGs or the LINC complex blocks Fascin's localization to the nuclear periphery. Furthermore, in cancer cells Fascin interacts directly with the LINC Complex. Here we show that loss of PGs results in delayed and aberrant border cell migration. Specifically, the border cell cluster elongates, with cells remaining attached to the anterior tip. These cells break off of the main cluster, and the remaining, intact cluster exhibits delayed migration. Current efforts are focused on determining the cell-autonomous vs non-autonomous roles of PGs in this migration. Importantly, global loss of LINC complex components or reduction of Fascin in the soma results in similar defects in migration. Genetic interaction studies will be used to assess PG regulation of Fascin and the LINC complex. This research is expected to provide the mechanistic insight into how PGs regulate 3D cellular migration by controlling actin binding proteins to modulate

the LINC complex, and, therefore, affect mechanotransduction. These findings will improve our understanding of the functions of PGs, Fascin, and the LINC complex, both developmentally and during tumor progression.

196A Hedgehog signaling modulates intercellular calcium waves through an incoherent feed-forward loop in the wing disc *Pavel Brodskiy*[1], Qinfeng Wu[1], Cody Narciso[1], Megan Levis[1,2], Jeremiah Zartman[1,2]. 1) Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN; 2) Bioengineering Graduate Program, University of Notre Dame, Notre Dame, IN.

Multi-cellular phenomena such as organ development require coordination among large cell populations. Intercellular Ca^{2+} waves (ICWs) have been described in many developmental contexts and represent a potential mechanism for long distance communication during morphogenesis and homeostasis. However, how ICWs are regulated during organ development is poorly understood. Here we have found that stimulation of ICW activity in wing discs specifically requires fly extract, the phospholipase C β1 (PLCβ1) isoform PLC21C, inositol-1,4,5-trisphosphate receptor (IP_3R, Itp-r83A) and gap junctions. We have developed a quantitative image analysis pipeline to show that ICWs in the *Drosophila* wing imaginal disc are spatiotemporally patterned, with higher amplitudes of oscillations in the posterior compartment. Further, patterning of ICWs depends upon the morphogenetic signaling activity of Hedgehog (Hh), which directs the anterior-posterior patterning of the wing disc. Modulation of ICWs by Hh signaling activity depends on both canonical transcriptional and noncanonical signaling that can be described as an incoherent feedforward loop. Thus, the dynamics of spontaneous ICWs are regulated by morphogenetic signaling and may provide an additional layer of organ-scale communication system for the developing wing disc.

197B Characteristics and differential spatio-temporal profiling of Shaggy protein isomers revealed by CRISPR genome engineering *Dagmara Korona*[1], Daniel Nightingale[2], Bertrand Fabre[2], Kathryn Lilley[2], Steven Russell[1]. 1) Genetics, University of Cambridge , Cambridge, United Kingdom; 2) Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.

The *shaggy* gene (*sgg*, *GSK-3*) encodes multiple protein isoforms with serine/threonine kinase activity that are key players in a number of diverse signalling systems. Sgg is involved in multiple events throughout *Drosophila* development, where dynamic changes occur at the level of the proteome[1]. There is some evidence that to mediate these various signalling events, different isoforms perform distinct functions[2] and consequently *sgg* is subject to extensive alternative splicing. To investigate the convergence of Sgg on various signalling pathways and the dynamics of spatio-temporal isomer localization, we applied CRISPR/Cas9 engineering technology to uniquely label several specific Sgg isoforms. For proteomic analyses, including pull-downs to identify protein interactors, we introduced a small StrepII-Flag-StrepII tag[3] via a removable Lox-3Px3_DsRed-Lox reporter. For tracking protein expression and localization during development we engineered yellow or red fluorescent protein fusions.

Interestingly, some Sgg isomers are reported to undergo a Caspase cleavage[2] important for aspects of CNS development. Using CRISPR/Cas9 we differentially labelled the termini of the cleaved exon with various tags. We also generated mutant version where we abolished the Caspase cleavage site to gain insights into the modulation of Sgg by non-apoptotic action of Caspases. Additionally, we generated transgenic flies harbouring tags fused to Sgg isomers that are predicted to have a non-canonical Valine start codon not shown to exist at the protein level. In summary, our extensive engineering of the *sgg* locus will allow us to confirm the localization of various Sgg isomers, examine functional redundancy between isomers, contribute to a better understanding of alternative splicing regulation and facilitate an analysis of the role of Sgg in different signalling pathways. Taken together, our pilot work on Sgg will not only set the scene for a further analysis of signalling pathways but also contribute to a better understanding of the *in vivo* proteomics of an important highly conserved regulatory protein.

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198C Building a functional regulator of Wg signaling: The β-catenin destruction complex *K. Schaefer*, C. Williams, M. Peifer. UNC-Chapel Hill, Chapel Hill, NC.

Wnt signaling is essential for proper development and tissue homeostasis. Over 85% of sporadic colorectal cancers contain a Wnt signaling activating mutation. Wnt signaling stabilizes β-catenin, which coactivates transcription of Wnt target genes. When Wnt is off, the destruction complex (DC), comprised of the tumor suppressor APC, scaffolding protein Axin, and kinases GSK3 and CK1, work together to phosphorylate β-catenin, thus labeling it for degradation. However, the structure of the DC and the mechanism by which it delivers phosphorylated β-catenin to the E3 ligase remain unanswered questions. Axin is believed to be the limiting factor in the complex, since in Xenopus egg extract Axin's protein levels were suggested to be more than a thousand-fold lower than any other protein in the complex, but this remains untested in vivo. In contrast, our RNA-seq data from fly embryos suggest that Axin and APC mRNA levels are similar throughout Drosophila larval development. We therefore are exploring the

structure, stoichiometry, and mechanism of the active DC, combining work in mammalian cell lines and Drosophila. We hypothesize that either total Axin levels or its ratio to APC2 determine whether an efficient DC is formed. To test this hypothesis, we are expressing GFP-Axin and/or GFP-APC2 at different levels using the UAS-GAL4 system and observing how varying these levels effect cell fate determination and β-catenin levels. Strikingly, we found that APC2 and Axin protein levels are within 10-fold of one another. When we over-express APC2, there is little effect on Drosophila embryo embryonic viability or cell fate. In contrast, relatively subtle Axin over-expression induces high levels of embryo mortality and dramatic changes[KS1] in cell fate, more similar to a *wg* null phenotype, suggesting Axin is a limiting factor in Wg signaling. We are also exploring how APC2 and Axin overexpression affect levels and localization of β-catenin. Axin contains a self-polymerization domain, allowing it to self-assemble into larger protein complexes. We now can observe these in living embryos after relatively low level Axin over-expression. We are currently exploring the effects of Wg signaling on Axin puncta localization and size. To compare the ratio of APC2 to Axin within the DC we are using two different fluorescent microscopy techniques, stepwise photobleaching and fluorescence comparison, to count the number of Axin and APC molecules within the DC. We hope these approaches will provide insight into the assembly and mechanism of action of the DC.

199A The exon junction complex regulates the splicing of cell polarity gene dlg1 to control Wingless signaling in development Min Liu, *Alan Jian Zhu*. School of Life Sciences, Peking University, Beijing, China.

Wingless (Wg)/Wnt signaling is conserved in all metazoan animals and plays critical roles in development. The Wg/Wnt morphogen reception is essential for signal activation, whose activity is mediated through the receptor complex and a scaffold protein Dishevelled (Dsh). In a genome wide RNAi screen, we find that the exon junction complex (EJC) activity is indispensable for Wg signaling by maintaining an appropriate level of Dsh protein for Wg ligand reception in Drosophila. Transcriptome analyses in Drosophila wing imaginal discs indicate that the EJC controls the splicing of the cell polarity gene discs large 1 (dlg1), whose coding protein directly interacts with Dsh. Genetic and biochemical experiments demonstrate that Dlg1 protein acts independently from its role in cell polarity to protect Dsh protein from lysosomal degradation. More importantly, human orthologous Dlg protein is sufficient to promote Dvl protein stabilization and Wnt signaling activity, thus revealing a conserved regulatory mechanism of Wg/Wnt signaling by Dlg and EJC.

200B Generating a new genetic tool for investigation of the requirements for *Mothers against dpp (Mad)* *Sheila Mosallaei*[1], Nick Rose[1,2], Laurel A. Raftery[1]. 1) School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) Current: University of Nevada, Reno School of Medicine, Reno, NV.

Cell communication via paracrine signaling plays an integral role in many cell processes, including differentiation, proliferation and migration. Signaling often involves transduction into the nucleus to regulate changes in gene transcription. In Drosophila, the BMP-activated transcription factor, Mad, has been manipulated to investigate signaling in many contexts, and is essential for many biological processes throughout all stages of development. From the available alleles, *Mad*^{10}and *Mad*^{12} are widely used as null alleles, based on the original characterization of maternal effect phenotypes. In particular, the C-terminal deletion of *Mad*^{12} prevents the receptor-mediated C-terminal phosphorylation, central for transduction of BMP signals. However, recent studies suggest that *Mad*^{10} and *Mad*^{12} retain a non-BMP-related function. Data from the lab of Ed Eivers (California State University, Los Angeles) suggest that mutant proteins produced by *Mad*^{10} or *Mad*^{12} can still associate with non-Smad binding partners, including Pangolin and Armadillo, components of the Wnt signaling pathway. This new evidence indicates there is no single gene *Mad* allele that completely lacks functional gene product. We are using CRISPR-*Cas9* to generate a deletion of the *Mad* coding region, and introduce an attP element for future engineering of modified and/or tagged Mad proteins. We expect that a molecular null will provide a reliable tool to assess the potential for Mad functions beyond the canonical BMP-activated Smad pathway. Funded by NSF 1355091.

201C The phosphatase Dullard dephosphorylates Mad to terminate BMP signaling *Hugo Urrutia*, Edward Eivers. Department of Biological Sciences California State University, Los Angeles 5151 State University Drive, Los Angeles, CA 90032.

Bone morphogenetic proteins (BMPs) are a family of growth factors that provide essential signals for proper embryonic development and adult tissue homeostasis. A crucial step in transducing intracellular BMP signals in *Drosophila* is the phosphorylation of the transcription factor, Mad at its C-terminal domain, by BMP receptor kinases. Controlling the intensity and range of BMP signals is essential during development and one known mechanism to regulate BMP signals is to phosphorylate Mad at three conserved linker domain serines by cyclin dependent kinase 8 (CDK8) and Shaggy. Linker phosphorylation results in Mad polyubiquitinylation and degradation by the proteasome. Here we present data demonstrating that the phosphatase Dullard is involved in dephosphorylating Mad at both its linker and C-terminal domains thus uncovering an alternative mechanism to terminate BMP signaling in *Drosophila*. We provide evidence that a hypomorphic Dullard allele or Dullard knockdown leads to elevated Mad phosphorylation levels, while Dullard overexpression resulted in reduced Mad

phosphorylations. Co-immunoprecipitation binding assays show phosphorylated Mad and Dullard physically interact, while mutation of Dullard's phosphatase domain still allowed Mad-Dullard interactions but abolished its ability to regulate Mad phosphorylations. In conclusion, we demonstrate that the phosphatase Dullard plays an important role in BMP signal regulation by dephosphorylating Mad, thus providing a potential mechanism to recycle Mad proteins for additional rounds of signaling.

202A The *Drosophila* xylosyltransferase Shams modulates the balance between Notch *cis*-inhibition and *trans*-activation by Delta. *Ashutosh Pandey*[1], Tom V Lee[1], Hamed Jafar-Nejad[1,2]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Program in Developmental Biology, Baylor College of Medicine, Houston, TX.

The *Drosophila* glucoside xylosyltransferase Shams xylosylates Notch and inhibits Notch signaling in specific contexts including wing vein development. However, the molecular mechanisms underlying context-specificity of the *shams* phenotype is not known. Considering the role of Delta-Notch signaling in wing vein formation, we hypothesized that Shams might affect Delta-mediated Notch signaling in *Drosophila.* Using genetic interaction studies, we find that altering the gene dosage of *Delta* affects the wing vein and head bristle phenotypes caused by loss of Shams or by mutations in the Notch xylosylation sites. Clonal analysis suggests that loss of *shams* promotes Delta-mediated Notch activation. Further, Notch *trans*-activation by ectopically overexpressed Delta shows a dramatic increase upon loss of *shams*. In agreement with the above *in vivo* observations, cell aggregation assays show that *shams* knock-down in Notch-expressing cells enhances the binding between Notch and *trans*-Delta without affecting the binding between Notch and *trans*-Serrate and cell surface levels of Notch. Loss of Shams does not impair the *cis*-inhibition of Notch by ectopic overexpression of ligands *in vivo* or the interaction of Notch and *cis*-ligands in S2 cells. Nevertheless, removing one copy of endogenous ligands mimics the effects of loss *shams* on Notch *trans*-activation by ectopic Delta. This favors the notion that *trans*-activation of Notch by Delta overcomes the *cis*-inhibition of Notch by endogenous ligands upon loss of *shams*. Taken together, our data suggest that xylosylation selectively impedes the binding of Notch with *trans*-Delta without affecting its binding with *cis*-ligands and thereby assists in determining the balance of Notch receptor's response to *cis*-ligands vs. *trans*-Delta during *Drosophila*development.

203B Tribbles interacts with Neuralized to regulate Notch signaling *Anna Shipman*, Leonard Dobens. School of Biological Studies, UMKC, Kansas City, MO.

Drosophila Tribbles (Trbl) is a pseudokinase with roles in key signaling pathways signaling pathways regulating cell migration, cell growth and cell differentiation. In a mis-expression screen for novel Tribbles interacting genes, Neuralized (Neur), an E3 ubiquitin ligase, was identified. Neuralized in involved in Delta ubiquitination and endocytosis to promote Notch signaling. Trbl misexpression effectively suppresses the wing notching phenotype associated with Neur misexpression and a physical interaction between Trbl and Neur can be detected by yeast two-hybrid assay. In the wing disc, Trbl misexpression effectively blocks scutellar bristle formation, consistent with opposing activity in Neur-expressing cells. Recently, it was shown in mouse that mammalian Tribbles 3 (Trb3) forms a complex with both the fly homolog of Mindbomb 1 (Mib1), an E3 ubiquitin ligase similar in structure and function to Neur, and the Fat Facets (Faf) homolog USP9x, a deubiquinating enzyme. Here we present data from ongoing work to further understand how interactions among Trbl, Neur, Mib1 and Faf promote Notch signaling.

204C An overexpression screen identifies genes that regulate intercellular signaling *Moe Wada*, Tomoko Tanaka, Yuka Doi, Hiroyuki Ishikawa. Grad. Sch. of Sci., Chiba Univ., Chiba, Japan.

In the process of intercellular signaling, numerous factors function extracellularly for sending, receiving, and modulating signals. To identify proteins that involves in cell-cell signaling extracellularly, overexpression screen was performed *in vivo.* Previous genome-wide RNAi screen in *Drosophila* identified 214 genes exhibiting planar cell polarity (PCP) defect by gene knock-down (Mummery-Widmer, Yamazaki et al., Nature, 2009). We evaluated those genes as a gene group for regulating cellular communication, and selected 63 genes that their translation products potentially function in extracellular. These genes were overexpressed by using *Gal4/UAS* system, and phenotypes by overexpression of the genes were analyzed.

As a result, we identified one gene exhibiting PCP defects by its overexpression in the adult wing. The fact both RNAi and overexpression of the gene led to PCP defects, indicating that this gene acts as a PCP regulator in the wing development. When epitope-tagged this protein was expressed in cultured S2 cells, its extracellular domain was secreted into the media. This raises the possibility that this protein regulates PCP in extracellular.

Further, we found that overexpression of another gene impaired Notch signaling in the adult thorax and wing. The expression of *wingless* at the dorsoventral compartment boundary of the wing disc, which depends on the activation of Notch signaling, was reduced by the overexpression of this gene in the wing discs. This indicates that this gene is implicated in Notch signaling. Notch accumulation was observed when the gene was overexpressed in the wing

imaginal discs. Moreover, overexpression of this gene enhanced phenotypes of *Notch* heterozygote in the adult wing. These results suggest that this gene negatively regulates Notch signaling.

205A An aberrant Notch signaling controls metabolic reprogramming during tumor formation *Cheng-Wei Wang*[1], Utpal Banerjee[1,2,3,4]. 1) Department of Molecular, Cell and Developmental Biology, UCLA, LA; 2) Department of Biological Chemistry, UCLA, LA; 3) Molecular Biology Institute, UCLA, LA; 4) Broad Stem Cell Research Center, UCLA, LA.

Notch signaling is known to contribute to the development of various human cancers. Based on current studies, the real role of the Notch pathway in human cancer is still a mystery. Recently, we have established a *Drosophila* glycolytic epithelial tumor model that exhibits most of oncogenic characteristics. We discovered that a single oncogene, Pvr (PDGF/VEGF-receptor) activation can trigger a sequential phosphorylation of the major kinases in ERK/MAPK, PI3K/Akt, and Src/JNK pathways to induce the metabolic shift and tumor formation. Interestingly, the protein expression of Notch, detected with an intracellular domain (N^{ICD}) antibody, is highly up-regulated in Pvr^{act}tumors. Transcription of E(spl) is a direct downstream target of Notch pathway in *Drosophila*. In Pvr^{act} induced tumor discs, in spite of high expression of Notch, not only is there no ectopic E(spl), even the wild type *E(spl)m8-GFP* expression is inhibited, suggesting that the wild type Notch signaling is inactivated. This suggests that what accumulates in Pvr^{act} cells is the full length Notch protein that is incapable of, and suppresses, canonical Notch signaling. By loss of function analysis, removing Notch from the tumor cells suppresses the accumulation of Sima protein and the transcription of LDH in Pvr^{act} tumors. This implies that Notch likely interacts with Sima to regulate glucose metabolism during tumor formation. We are currently investigating which potential pathway induces Notch accumulation and how this aberrant Notch signaling controls metabolic reprogramming in our *Drosophila* tumor model.

206B A feedback loop linking Notch signaling and epigenetic silencing Juan Du, Tao He, *Alan Jian Zhu*. School of Life Sciences, Peking University, Beijing, China.

The activities of developmental signaling are controlled by a large array of post-translational modification events. By contrast, very little is known about the mechanisms that regulate the expression of their core pathway components. In a genetic screen, we identified a novel *Drosophila* gene, *stuxnet*, that functions as a key component of Notch signaling, a process at the core of cell fate decisions in development, adult tissue homeostasis and cancer. We showed that *stuxnet* is both necessary and sufficient for transcription of the *Notch* receptor gene in the wing imaginal disc. Genetic interaction analyses revealed that *stuxnet* lethal mutation can be rescued by reducing the activity of Polycomb (Pc), an essential component of the Polycomb Repressive Group complex 1 (PRC1) that is known to epigenetically silence target genes critical for animal development. Indeed, Notch locus contains a Polycomb Response Element (PRE). Our ChIP analysis with Pc and *Notch* PRE-GFP reporter assays in wing discs confirmed this is a *bona fide* PRE responsible for PRC1-mediated *Notch* gene silencing. Mechanistically, Stuxnet protein physically interacts with and subsequently destabilizes Pc protein *in vivo*. Thus, Stuxnet facilitates Notch signaling by destabilizing the PRC1 complex, thereby reducing the repressive chromatin modification marker on the *Notch* locus. Intriguingly, *stuxnet* may serve as a target of Notch signaling, establishing a feedback loop between Notch signaling and PRC1-mediated epigenetic activity to maintain a proper level of Notch receptor expression in development.

207C Hipk induces tumorigenesis in Drosophila Jessica Blaquiere, Nathan Wray, *Esther Verheyen*. Molecular Biology & Biochemistry, Simon Fraser Univ, Vancouver, BC, Canada.

Signal transduction pathways are crucial for coordinated development and growth of multicellular organisms. Dysregulation and mutations of components in these pathways can often lead to tumourigenesis. The evolutionarily conserved Homeodomain-Interacting-Protein-Kinase (Hipk) is a potent growth regulator and modulator of numerous conserved signaling pathways. Elevated levels of Hipk in Drosophila lead to tumour-like masses resembling those found in fly models of leukemia. We are investigating if Hipk induces tumours through a combinatorial mode. We are determining the underlying mechanisms through which Hipk can induce tumours using genetic interaction studies and molecular markers. A prime candidate for contributing to Hipk-induced tumorigenesis is the JAK/STAT signaling pathway. A point mutation similar to those seen in human blood cancers in the Drosophila Janus kinase (called *hop*) causes constitutive activation of the JAK/STAT pathway and results in blood cell tumours in larval and adult stages. We investigated whether Hipk causes tumours through JAK/STAT. First we show that elevated Hipk in blood cells phenocopies effects seen with the hyperactive form of Hop. Furthermore, Hipk induces enhanced proliferation of hemocytes and the effects of Hipk are kinase dependent. We find that reduction of Hipk can suppress the tumorigenic effects of activated Hop. RNAi against Hipk in hemocytes can suppress both the timing of lethality and the tumour load in activated *hop* flies. Furthermore, we find that Hipk is required for endogenous JAK/STAT pathway activity, since homozygous mutant tissue shows markedly reduced expression of a STAT reporter. To investigate the mechanism underlying this interaction, we performed a proximity ligation assay (PLA) between Hipk and STAT92E,

the Drosophila STAT. Cells co-expressing Hipk and STAT92E show a robust PLA signal indicating an interaction. We are currently confirming this through in vitro and in vivo binding studies. Our work shows that Hipk is required for JAK/STAT signaling during normal development and in fly blood cancer. Future work will determine if Hipk's ability to promote other pathways contributes to blood cell tumours.

208A A *miR-285*-Yki/Mask double-negative feedback loop mediates blood-brain barrier integrity in *Drosophila* Dong Li[1,2], Yanling Liu[1,2], Chunli Pei[3], Peng Zhang[3], Linqing Pan[2], Jing Xiao[4], Songshu Meng[2], Zengqiang Yuan[3], *Xiaolin Bi*[1,2]. 1) Department of Biological Sciences, College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, China; 2) Institute of Cancer Stem Cell, Cancer Center, Dalian Medical University, Dalian 116044, China; 3) State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China; 4) Department of Oral Basic Science, College of Stomatology, Dalian Medical University, Dalian 116044, China.

Hippo signaling pathway is highly conserved from *Drosophila* to mammals and plays a central role to maintain organ size and tissue homeostasis. The blood-brain barrier (BBB) physiologically isolate brain from circulating blood or hemolymph system and its integrity is strictly maintained to perform sophisticated neuronal functions. Till now, the underlying mechanisms of subperineurial glia (SPG) growth and BBB maintenance during development are not clear. Here, we report a novel *miR-285*-Yki/Mask double-negative feedback loop which can regulate SPG growth and BBB integrity. Flies loss or over-expression of *miR-285* have aberrant SPG ploidy and disruptive septate junctions of BBB. Mechanistically, *miR-285* directly target Yki cofactor Mask to suppress Yki signaling to downstream target *cyclin E*, a key regulator of DNA replication. Disturbance of *cyclin E* expression leads to abnormal endoreplication in SPG which causes unusual ploidy and defective septate junction. Moreover, expression of *miR-285* is increased by knockdown of *yki* or *mask*, while decreased with *yki* overexpression, thus forming a double-negative feedback loop. This regulatory loop is crucial for sustaining appropriate Yki/Mask activity and *cyclin E* level to maintain SPG ploidy and BBB integrity. Perturbation to this signaling loop, either by abnormally changed *miR-285* level or Yki activity, causes irregular SPG ploidy and thus break down BBB. Furthermore, *miR-285* promotes canonical Hippo pathway mediated apoptosis independent of p53 or JNK pathway. Collectively, we identify an exquisite regulatory mechanism for BBB maintenance, which is through fine-tuned DNA polyploidy by *miR-285*-Yki/Mask feedback loop.

209B Role of ubiquitination in trafficking of Fat signaling pathway components *Jyoti Misra*, Kenneth Irvine. Waksman Institute, Rutgers University, Piscataway, NJ.

Proper coordination of growth and morphogenesis during development is critical to the formation of organs of appropriate size and shape. The evolutionarily conserved protocadherins, Dachsous (Ds) and Fat constitute a signaling pathway that coordinates growth and morphogenesis by regulating the Hippo pathway and planar cell polarity (PCP) respectively. The atypical myosin, Dachs is a key downstream effector of Fat signaling that mediates both of these effects. Fat regulates growth and PCP by modulating the levels and polarity of Dachs at the apical membrane. Recently, we identified the SH3 domain containing adapter protein, Vamana (Vam) as a novel downstream effector of this signaling pathway. Vam plays a critical role in membrane localization of Dachs. Further, it functions as an adapter by physically connecting Dachs to Ds and Fat intra cellular domains. In order to identify additional regulators of this signaling pathway, we have conducted a genetic screen using RNAi targeted against the ubiquitin ligases encoded by the *Drosophila* genome and have isolated a novel E3 ubiquitin ligase, which when depleted exhibits short cross vein spacing in the adult wings, a phenotype reminiscent of mutations in Fat signaling pathway. Cells mutant for this ubiquitin ligase have significantly higher levels of Fat, Dachs and Vam at the subapical membrane. In contrast, cell that overexpress this ligase have decreased amounts of apical Dachs and Vam. Interestingly, overexpression of a dominant negative version of this enzyme results in accumulation of Dachs and Vam in bright punctate structures in the cytoplasm, suggesting that it is required for their proper trafficking. Our current efforts are aimed at identifying the mechanism by which it regulates trafficking of these proteins.

210C *In vitro* and *in vivo* Yki protein interactome in *Drosophila melanogaster* *Heya Zhao*[1], Can Zhang[2], Dennis Zeh[3], Daniel Stokes[1], Alexander Letizia[1], Jason Evans[3], Kenneth Moberg[2], Alexey Veraksa[1]. 1) Department of Biology, University of Massachusetts Boston, Boston, MA; 2) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA; 3) Department of Chemistry, University of Massachusetts Boston, Boston, MA.

The Hippo pathway plays a key role in controlling organ growth via the inhibition of a transcriptional effector Yorkie (Yki). Dysregulation of Hippo signaling results in abnormal cell proliferation and tissue overgrowth. Although previous studies have revealed a number of Yki-interacting proteins, comprehensive understanding of the *in vivo* Yki protein interactome and its response to Hippo pathway activation remains elusive. To systematically study the Yki protein interactome, we carried out affinity purification-mass spectrometry (AP-MS) of Yki protein complexes in *Drosophila* S2 cells and embryos. In support of the soundness of our approach, we identified all of the core Hippo pathway components and Yki interactors, including Hippo, Warts and Scalloped. In addition, we identified multiple

novel proteins putatively associated with Yki, and confirmed the *in vitro* and *in vivo* interactions between Yki and these proteins in genetic and biochemical assays. Our data suggest previously unappreciated connections between Yki and cytoplasmic cytoskeletal and endocytic regulators, as well as nuclear components involved in the regulation of transcription. Furthermore, we quantitatively compared the proteomic profile of wild type Yki to the interactomes of its activating or deactivating mutant isoforms, and observed mutant-specific, enhanced or reduced interactions, suggesting potential new mechanisms of regulating Yki activity. In summary, our study of the *in vitro* and *in vivo* Yki protein interactome provides a comprehensive view of the Yki protein interaction network. We expect that subsequent mechanistic studies of the Yki interactome and its alterations in the mutants will provide further insights into the regulation of Yki function in both physiological and pathological conditions.

211A Ras is Required for Toll Signalling in the Drosophila Embryo *Jay B. Lusk*[1], Vanessa Lam[1], Nicholas S. Tolwinski[1,2]. 1) Yale-NUS College, Division of Science, Singapore; 2) National University of Singapore, Department of Biological Science, Singapore.

The *toll* and EGF pathways are critical for early embryonic patterning, and have great clinical significance for human pathologies. Therefore, we set out to understand how the *toll* and EGF pathways are linked, and focused our analysis on Raf/Ras's connection to the *toll* pathway. We utilized a Raf neomorph which truncated the second and third conserved regions of Raf (the negative regulatory domains and kinase site), leaving only the first conserved region, which binds to Ras. We performed anti-*dorsal* stains on this Raf neomorph, which showed extreme dorsalization of the embryo. We crossed this mutant with a constitutively active *toll* mutant (i.e. extremely ventralized) which resulted in a partial rescue. We looked at the localisation of this Raf mutant, and surprisingly found it to be in the nucleus. As this mutant binds Ras tightly, we concluded that Ras was sequestered by Raf away from its normal plasma membrane localisation preventing Ras from acting in a required Toll pathway signal transduction step. We propose that Ras is a necessary component of the Toll pathway during early embryonic patterning.

212B Distinct Transcriptional Mechanisms Account for the Autonomous and Nonautonomous Inhibition of Growth Induced by Fat Body Toll Signaling. *Nigel Muhammad*, Miyuki Suzawa, Michelle Bland. Department of Pharmacology, University of Virginia, Charlottesville, VA.

In *Drosophila*, Toll signaling is activated in response to Gram-positive bacterial or fungal infection and drives synthesis of anti-microbial peptides (AMPs), that carry out the humoral arm of the immune response. Dif is a NF-kB homolog that acts downstream of Toll to drive AMP expression. Activation of fat body Toll signaling not only induces AMPs, but also leads to insulin resistance and concomitant reductions in both fat body cell size and whole-animal growth. Both phenotypes are rescued by expression of constitutively-active Akt, underscoring the importance of insulin signaling in driving growth. We find that Dif is required downstream of Toll activation to suppress growth locally and globally. We used a mosaic approach to drive transgene expression in fat body cells and found that expression of a constitutively-active Toll ($Toll^{10b}$) results in a 42% decrease in cell size. Knocking down Dif in cells expressing $Toll^{10b}$ results in a 8% decrease in cell size, a phenotype that is nearly indistinguishable from wild type. We used r4-Gal4 to drive transgenes throughout the fat body and found that Toll also requires Dif to inhibit whole animal growth. We next asked whether Dif is sufficient on its own to block growth. $Toll^{10b}$ or Dif overexpression in fat body led to equivalent AMP levels, indicating that both transgenes are capable of driving canonical pathway responses. Similar to results with $Toll^{10b}$, overexpression of Dif alone in fat body clones led to a 22% decrease in cell size. However, overexpression of Dif throughout the fat body did not block whole-animal growth. The transcriptional role of Dif – necessary but not fully sufficient – in growth processes inhibited by Toll signaling underscores the distinction between autonomous and nonautonomous growth control in the fat body. Our data suggest that other transcription factors or Dif post-translational modification in response to Toll activation is required to induce distinct sets of genes that modulate fat body cell growth and whole-animal growth. Dorsal is another NF-kB homolog that acts downstream of Toll. We find that overexpression of dorsal throughout the fat body reduces whole animal growth. However, we did not find a decrease in cell size when dorsal was overexpressed in fat body clones. This suggests that dorsal may play a role in the cell-nonautonomous regulation of growth by Toll signaling. Finally, identification of the genes induced by Toll signaling in the fat body will shed light on how this pathway transcriptionally regulates growth autonomously in the fat body and nonautonomously in the periphery.

213C Somatic activation of Rolled/ERK, downstream of EGFR, synchronizes spermatogonial proliferation in *Drosophila* testis *Samir Gupta*, Shambhabi Chatterjee, Bhavana Varshney, Krishanu Ray. Tata Institute of Fundamental Research, Mumbai, India.

Transit amplification of progenitor cells maintains tissue homeostasis through optimization of proliferation and differentiation. In *Drosophila melanogaster* testis, the proliferation of the germline stem cell progeny is tightly regulated by factors present in both germline and neighboring somatic cells. Although the EGFR activation in the somatic cyst cells has been reported to control spermatogonial divisions, the underlying cellular and molecular mechanisms are still unclear. Here, we report the results of a candidate screen of the EGFR downstream, and their

quantitative impact on the spermatogonial proliferation. We show that - (a) the activation of the ERK/MAPK cascade in the soma is essential for arresting spermatogonial divisions after four cycles; (b) the somatic Rho/Rac network is redundant for the regulation of spermatogonial divisions; (c) the process acts independently of the bag-of-marble expression in germline cells; and (d) somatic encapsulation around spermatogonia remains intact upon ERK/MAPK downregulation. Furthermore, somatic loss of either EGFR or ERK disrupted the otherwise synchronized mitotic divisions of germline cells within a cyst. Together, these results suggest that somatic activation of a specific EGFR downstream synchronizes the divisions of germline cells, which is essential for the termination of transit amplification after four cycles.

214A Germ cell transit amplification is non-autonomously regulated by the EGFR downstream target, miRNA bantam, in *Drosophila* testis *Chetanchandra S Joshi*, Krishanu Ray. Department of Biological Sciences, Tata Institute of Fundamental Research, Colaba, Mumbai, India.

Epidermal Growth Factor Receptor (EGFR) signaling is essential for establishing cell-fate and maintaining tissue homeostasis. Dysregulation of the signaling cascade leads to uncontrolled cell growth and malignancy.
In *Drosophila* wing primordium, EGFR activates the miRNA *bantam*, an anti-apoptotic and pro-proliferative agent which plays a major role in the epithelial to mesenchymal transition (EMT). Here we show that the bantam miRNA also plays a significant role in controlling the germ cell proliferation in *Drosophila* testis. In *Drosophila* testis, periodic and transient activation of EGFR signaling in the surrounding somatic cyst cells attenuates the transit amplifying divisions of the enclosed germline. Using bantam sensor, we found that the miRNA is exclusively expressed in somatic cyst cells during transit amplification stages and its expression overlaps with the phases of phosphor-ERK, a downstream effector of EGFR signaling. Somatic over-expression of bantam led to an increase in the germ cell number, whereas expression of bantam-sponge reduced the number of germ cells. The latter appeared to be arrested in the G1 phase of the cell cycle. These results suggest a novel cell non-autonomous role of the miRNA bantam in regulating the germline divisions in *Drosophila* testis.

215B Search for a novel small molecule inhibitor of PLCγ *Chitra Naidu*, Michelle Latino, Claire Rosenwasser, Todd Rosenberg, 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 R7 photoreceptors in ~60% ommatidia. Our objective is to identify a novel small molecule inhibitor of PLCγ using *Drosophila* as a model system.

In a 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 Sl function. Thus, drug fed MS1096>Aos flies were used as a sensitive model system to detect potential Sl inhibition by looking for L3 vein recovery. 37 of the 1,596 small molecules, provided by the NCI, showed significant results.

In a secondary screen, we confirmed EGFR inhibition by looking at photoreceptor differentiation in the eye in an sl^7 mutant. sl^7 is a missense mutation that results in 5-10% ommatidia with extra R7 photoreceptors. Further inhibition of Sl or the EGFR pathway would result in a higher percentage of R7 recruitment. So far, we have identified 9 small molecules as potential inhibitors.

We are currently in the process of retesting these 9 molecules using the MS1096>Aos flies on a larger scale. Subsequent experiments will try to determine whether any of the small molecules identified do in fact inhibit Sl.

216C Capicua preferentially binds to dually phosphorylated ERK by recognizing altered conformation of the hydrophobic pocket of the ERK DRS domain *Sayantanee Paul*, Liu Yang, Alexey Veraksa. Department of Biology, University of Massachusetts Boston, Boston, MA 02125, USA.

The extracellular signal-regulated kinase (ERK) pathway is an essential signaling component of developmental programs in *Drosophila* and other metazoans. As a final element of the Raf-MEK-ERK kinase cascade, ERK phosphorylates transcriptional regulatory proteins, resulting in a fine-tuned spatio-temporal expression of target genes. *Drosophila* ERK Rolled (Rl) interacts with the transcriptional repressor protein Capicua (Cic) during development to control tissue patterning and cell proliferation. Cic repressor activity is antagonized by ERK, however the molecular mechanisms of the ERK mediated derepression of Cic target genes remain elusive. Recent reports

suggested that phosphorylation by ERK leads to a rapid downregulation of Cic repressor activity in the nucleus, followed by its slower translocation to cytoplasm, where it eventually gets degraded. Using *in vivo* and *in vitro* biochemical assays, we have established that Cic preferentially binds dpERK (dually phosphorylated ERK) with a much higher affinity than unphosphorylated ERK. This interaction is mediated by the C2 domain in Cic that was previously shown to bind ERK, but whose importance in discrimination between ERK and dpERK has not been reported. Our mutagenesis/binding experiments suggest that ERK phosphorylation in the activation loop induces a conformational change in the hydrophobic pocket of the ERK D-recruitment site (DRS, alternatively known as the common docking, or CD domain), which enhances its binding to the C2 motif and thereby allows Cic to discriminate between ERK and dpERK. Our results elucidate a molecular mechanism in which the difference in the affinity of Cic for dpERK vs. ERK would allow a transient pulse of ERK activation to establish gene expression patterns at fast timescales *in vivo*.

217A Characterization of novel epidermal growth factor receptor target genes implicated in *Drosophila* development *Sergey Svintozelskiy*, Alexis Morgan, Nathan Seabridge, Zachary Walter, Lisa Kadlec. Dept. of Biology, Wilkes University, Wilkes-Barre, PA.

Signaling by the *Drosophila* epidermal growth factor receptor (Egfr) plays important roles in many aspects of development, including oogenesis, embryogenesis and proper development of both the eye and the wing. For example, in the wing Egfr signaling is involved in vein tissue specification, and in the ovary the pathway is known to play key roles in the establishment of the body axes during oogenesis. Microarray screens by our lab and others have been used to identify potential downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression using fly ovaries in which the activity of the Egfr-pathway was reduced (*grk* mutant), normal (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. *In situ* hybridization was used to look at mRNA localization of target genes, and UAS-RNAi was used to knock down expression in either specific tissues or ubiquitously via a tubulin driver. Several target genes exhibited developmentally regulated ovarian expression. Gene knockdown phenotypes included decreased eggshell integrity and in at least one case for Gal4-tubulin driven RNAi, pupal lethality. We are currently extending our studies by utilizing the UAS/Gal4 system to perform a functional screen of previously identified, but untested, target genes whose expression is highly upregulated in response to constitutive Egf receptor activity. In particular, the available libraries of UAS-RNAi transgenic flies are being used in concert with ovarian and other Gal4 driver lines to identify additional genes with roles in *Drosophila* developmental events. We have identified several additional genes which appear to have roles in normal eggshell formation. RT-PCR has confirmed the up-regulation of some of our newly identified targets, and we are further investigating fertility of the knockdown flies as well gene expression patterns in situ. We additionally plan to look at the effects of ubiquitous gene knockdown as well as of P-element insertion mutations where available.

218B The “gatekeeper” function of *Drosophila* Seven-in-Absentia (SINA) E3 ligase and its human homologs, SIAH1 and SIAH2, is highly conserved for proper RAS signal transduction *Robert E. Van Sciver*[1], Yajun Cao[1], Atique U. Ahmed[2], Amy H. Tang[1,2]. 1) Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, VA; 2) Department of Surgery, Mayo Clinic, Rochester, MN 55905.

Seven-IN-Absentia (SINA) is an evolutionarily conserved E3 ubiquitin ligase that is the most downstream signaling module identified in the RAS pathway. Underscoring the importance of SINA is its high evolutionary conservation with over 83% amino acid identity shared between *Drosophila* SINA and human SINA homologs (SIAHs). As a major signaling “gatekeeper” in the RAS pathway, we have shown that SIAH is required for oncogenic K-RAS-driven tumorigenesis and metastasis in human pancreatic, lung and breast cancer. Since SIAHs appear to be the ideal drug target to inhibit “undruggable” K-RAS activation, it is important to delineate the activity, regulation, and substrate targeting mechanism(s) of this highly conserved family of SINA/SIAH E3 ligases. To delineate SINA function, we performed an F1 modifier screen using ethyl methanesulfonate (EMS) and X-ray radiation, isolating 28 novel *sina* mutant alleles. These mutant alleles exhibit much stronger mutant phenotypes than those of the previously published *sina*^{2} and *sina*^{3} alleles, suggesting that the *sina*^{2} and *sina*^{3} alleles are hypomorphic alleles. Sequencing analysis of these *sina*^{mutant} alleles reveals the functional roles of mutated residues and protein domains. In addition, we have generated a complete panel of transgenic fly lines expressing either wild-type (WT) or proteolysis-deficient (PD) SINA/SIAH under control of UAS. The corresponding *UAS-sina/siah*$^{GOF/LOF}$ phenotypes have been characterized using *sev*-, *GMR*-, *dpp*- and *salivary gland*-GAL4 drivers to elucidate the developmental outcomes of altered SINA/SIAH expression upon RAS activation. Ectopic expression of *sina*$^{WT/PD}$/*siah*$^{WT/PD}$ in neurons resulted in dramatic changes in neuronal cell fate in the developing eye and notum, causing PNS neurodegenerative phenotypes. Our results show that the biological functions of fly SINA and human SIAH1/SIAH2 are evolutionarily conserved and functionally interchangeable. Mechanistic insights and regulatory principles learned from *Drosophila* can be directly

applied to cancer biology to develop and validate next-generation anti-SIAH-based anti-K-RAS and anticancer therapy in the future.

219C Sequential Activation of Pointed Initiates Oenocyte Specification *Guolun Wang*, Lisa Gutzwiller, Brian Gebelein. Cincinnati Children's Hospital Medical Center, CINCINNATI, OH.

In *Drosophila*, larval oenocytes are hepatocyte-like cells that are primarily involved in lipid metabolism. Larval oenocytes are specified during embryonic development by an EGF signal secreted from a select subset of sensory organ precursor cells in abdominal segments. Here we show that the major downstream transcription effector protein Pointed (Pnt) plays an essential role in oenocyte specification. The *pnt* gene uses different promoters to encode two ETS-domain containing isoforms, PntP1 and PntP2. In oenocyte precursor cells, we found that both PntP2 and PntP1 are sequentially activated and both isoforms are essential for oenocyte development. We identified an evolutionarily conserved 700bp cis-regulatory module (POE) within the pointed locus that activates oenocyte-specific expression in transgenic reporter assays. We also characterized a MIMIC transposon insertion within POE enhancer that disrupts oenocyte development, and complementation analysis revealed that POE is a PntP1-specific enhancer. Mutation analysis of POE enhancer demonstrated that the abdomen-specific Hox factor Abdominal-A (Abd-A) and the cofactor complex of Extradenticle and Homothorax play crucial roles in maintaining the appropriate activity of POE enhancer. In addition, POE also has several ETS binding sites that are required to ensure the robust oenocyte expression. Taken together, our data support a model whereby EGF signals lead to the phosphorylation of PntP2, and then the POE enhancer integrates PntP2 and Abd-A Hox complexes to activate PntP1 during oenocyte specification.

220A Nutrient regulated spargel/dPGC1 expression is essential for *Drosophila* oogenesis *MOHAMMAD Abul BASAR*, Kishana Williamsons, Atanu Duttaroy. BIOLOGY, HOWARD UNIVERSITY, WASHINGTON, DC.

Genetic epistasis analysis has revealed that spargel/dPGC influences Insulin-Tor mediated cell growth by acting downstream to S6K. Moreover, spargel hypomorphic condition negatively affects the female fecundity where ovarian egg chambers grow much slowly into mature oocytes. In response to available nutrients, *Drosophila* adjusts its ovarian growth rate through Insulin signaling pathway. In female adults, spargel expression is overtly limited to the ovaries in comparison to rest of the body indicating that spargel plays an essential role in nutrient coordinated ovarian growth through insulin signaling. Since spargel is primarily expressed in the ovary, we asked whether varying nutrient condition would influence spargel expression in the ovary or not? Indeed, the ovarian spargel expression drops significantly when flies are transferred from high nutrient to no nutrient diet. Female germline-specific knockdown of *spargel* completely shuts down the egg chamber’s growth at mid-stage, where they eventually die through caspase-3 activation. Most interestingly, supplying excess nutrient to the fly doesn’t change *spargel/dPGC-1* knockdown ovarian phenotype, which proves that spargel serves as a critical facilitator of nutrition and reproduction. The co-accumulation of Rab4 and Notch in *spargel* depleted nurse cells indicated that spargel might play a potential role in endocytic molecular trafficking to modulate cellular function. Finally, we now established that the RNA Recognition Motif (RRM) of spargel plays an essential role in delivering spargel mediated female fertility.

221B Structure-function analysis of β-arrestin Kurtz reveals its role in epithelial morphogenesis as a regulator of the Fog-Mist signaling pathway *F. Chai*[1], T. Musoke[1], G. Tarabelsi[1], A. Veraksa[1], S. Rogers[2]. 1) Department of Biology, University of Massachusetts Boston, Boston, MA 02125, USA; 2) Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.

β-arrestins are key regulators of signaling via the G protein coupled receptors (GPCRs), serving both as signal terminators and transducers. Previous studies, primarily performed in mammalian cultured cells, identified various structural elements in β-arrestins that were shown or predicted to affect their function. However, the importance of these elements in vivo is still unclear, and the developmental roles of β-arrestins are not well understood. Kurtz (Krz) is the single ortholog of mammalian β-arrestins in the *Drosophila* genome. We found that the Krz-KKVL/A mutant that is defective in both the GPCR-phosphosensing and receptor-binding finger loop regions acts as functional null in vivo. Endosome recruitment assays in S2 cells revealed that the Krz-KKVL/A mutation completely abolishes the GPCR-binding ability of Krz. Remarkably, *krz* maternal mutant embryos exhibited gastrulation defects that were similar to those observed in embryos with hyperactive Fog-Mist signaling. The GPCR Mist is activated by its ligand Folded gastrulation (Fog) and is responsible for cellular contractility and epithelial morphogenesis in *Drosophila* development. Genetic analysis demonstrated that Krz function is necessary for limiting the activity of Fog-Mist signaling during different developmental stages. Our results have revealed the structural elements in β-arrestin Krz that are essential for its function as the GPCR regulator, and uncovered a critical role of Krz in controlling Fog-Mist signaling and epithelial morphogenesis in *Drosophila* development.

222C Improving the Molecular Toolkit to Study Muscle Differentiation *Emily Czajkowski*[1], Richard Cripps[1], Anton Bryantsev[2]. 1) Department of Biology, University of New Mexico, Albuquerque, NM; 2) Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

During myogenesis, multinucleate muscle fibers arise from the fusion of single nucleate myoblasts. In Drosophila, myoblast fusion is initiated by a specific kind of myoblast called founder cells (FCs), which determine the differentiation program for the nascent muscle fibers. Much of the information about muscle fusion and general muscle development is obtained via a toolkit of genetic drivers that are used to manipulate gene expression in a cell-specific manner. rP298 is a FC-specific genetic driver, but its practical use is rather limited because it quickly becomes inactive upon the first fusion between FCs and myoblasts. We demonstrate that by adding a self-activating transgene UAS-Gal4 to rP298, it is possible to prolong Gal4 expression beyond myoblast fusion, into more advanced developmental stages including adulthood. The idea is that Gal4, once initiated by rP298, activates itself though the UAS promoter and maintains continuous Gal4 expression. Thus, by allowing the Gal4 gene to maintain expression in post-fused muscle fibers, we now can study those regulatory genes that only become activated in FCs and which are likely to control the muscle differentiation program.

223A Defining the interactions of Aret and Vasa in muscle fiber specific alternative splicing *Sandy Oas*[1], Anton Bryantsev[2], Richard Cripps[1]. 1) University of New Mexico, Albuquerque, NM; 2) Kennesaw State University, Kennesaw, GA.

We have used adult flies to understand the genetic mechanisms determining differentiation of somatic muscles into specific fiber types. *Drosophila* flight and jump muscles are distinct functionally and biochemically. This distinction is due to differentially expressed genes and differentially spliced mRNA transcripts unique to each muscle type. From our previous work, we showed that *aret* is an important and novel alternative splicing regulator in the adult fly musculature. Endogenous Aret protein is localized to the nuclei of the flight muscles within the thorax, and promotes flight muscle patterns of alternative splicing. Without this regulator, the flies are incapable of flight due to ultra-structural changes within the flight muscles. Ectopic expression of Aret in the jump muscle and in cultured cells promotes flight muscle specific splicing. This indicates Aret works autonomously as a regulator of alternative splicing in flight muscles. In search for interactive partners of Aret, we describe a potential role for the DEAD box RNA helicase Vasa in *Drosophila* muscle development. Aret and Vasa co-localize in the adult flight muscles, and knockdown of Vasa leads to cytoplasmic localization of the Aret protein. Vasa have been previously shown to bind to Importin α2 (Kpna2) which functions as a shuttle for proteins between the nucleus and cytoplasm, therefore we hypothesize that Vasa may function in a similar manner with Aret, by shuttling Aret from the cytoplasm back to the nucleus. The Aret mammalian orthologs, the CELF proteins, are implicated as regulators of alternative splicing, and improper dosages result in cardiomyopathies and muscular dystrophies. In contrast, the mammalian Vasa have not been implicated in muscle development, and this study suggests a novel role for Vasa in muscle development.

224B *canB2*, a calcium binding subunit of Calcineurin, is required for maintaining calcium homeostasis in indirect flight muscles of Drosophila *Ruchi Jhonsa*, Upendra Nongthomba. MRDG, Indian Institute of Science, Bangalore, India.

Muscle contraction involves sliding of thick and thin filaments to generate force and is majorly regulated by calcium. In the present study, we report the functional characterization of a calcium binding protein 'Calcineurin' that is involved in skeletal and cardiac muscle hypertrophy in humans. Using the indirect flight muscles (IFMs) of the *Drosophila*, we are trying to understand how calcineurin may be involved in pathogenesis of muscle hypertrophy in vertebrates. Our study shows that the loss of Calcineurin-A isoforms in the IFMs, does not lead to structural or functional impairments, suggesting a functional redundancy between different Calcineurin-A isoforms. Several lines of evidence demonstrate the importance of regulatory subunit, Calcineurin-B (CanB2), in regulation of Calcineurin-A phosphatase activity in IFMs. Reducing the levels of *canB2,* regulatory subunit of Calcineurin, leads to muscle hypercontraction phenotype, which is characterized by extensive muscle thinning and tearing. This phenotype has been previously reported for mutants of structural proteins like Troponin I ($wupA^{hdp-2}$, $wupA^{hdp-3}$) and Troponin T (up^{1}, up^{101}). Genetic interaction between the Calcineurin-B and hypercontracting alleles of troponin show enhancement in the hypercontraction phenotype of up^{101} mutant along with complete loss of muscles in majority of the flies. We believe that CanB2 being a calcium binding protein can act as a sensor for regulation of calcium homeostasis in muscles and reduction in the levels of Calcineurin may perturb a fine balance between cytosolic calcium concentration and contraction. Furthermore, up^{101} is a calcium sensitive mutation and affects the contractile properties of the troponin complex by reducing the threshold concentration of calcium required for contraction. We show that null mutants of *canB2* have range of abnormalities in spontaneous calcium spiking including slow decay time, and abnormal spike broadening. Additional time that calcium ions spends in the cytosol results in irregular muscle contraction that gets further enhanced in a troponin mutant background (up^{101}), which is primed to contract at calcium concentrations lesser than the threshold. This observation was further supported by genetic study wherein increase in the levels of calcium in *canB2* knockdown background enhanced the severity of hypercontraction

phenotype. In conclusion, these results reflect an important role of *canB2* in maintaining calcium homeostasis in muscles.

225C Histamine Recycling Is Mediated by CarT, a Carcinine Transporter in *Drosophila* Photoreceptors *Ying Xu*. National Institute of Biological Sciences,Beijing, Beijing, China.

Histamine is an important chemical messenger that regulates multiple physiological processes in both vertebrate and invertebrate animals. Even so, how glial cells and neurons recycle histamine remains to be elucidated. *Drosophila* photoreceptor neurons use histamine as a neurotransmitter, and the released histamine is recycled through neighboring glia, where it is conjugated to β-alanine to form carcinine. However, how carcinine is then returned to the photoreceptor remains unclear. In an mRNA-seq screen for photoreceptor cell-enriched transporters, we identified CG9317, an SLC22 transporter family protein, and named it CarT (Carcinine Transporter). S2 cells that express CarT are able to take up carcinine *in vitro*. In the compound eye, CarT is exclusively localized to photoreceptor terminals. Through the HPLC test, we found that null mutations of *cart* alter the content of histamine and its metabolites. Moreover, the ERG and behavior results showed that null *cart* mutants are defective in photoreceptor synaptic transmission and lack phototaxis. These findings reveal that CarT is required for histamine recycling at histaminergic photoreceptors and provide evidence for a CarT-dependent neurotransmitter trafficking pathway between glial cells and photoreceptor terminals.

226A Adult muscle formation requires *Drosophila* importin-7 for proliferation of wing disc-associated muscle precursors *Kumar Vishal*, Samantha Gameros, Marta Stetsiv, David Brooks, Erika Geisbrecht. Kansas State University, 141 Chalmers Hall, Manhattan KS 66506.

A small number of adult muscle precursor (AMP) cells are set aside in the embryo and eventually give rise to the *Drosophila* indirect flight muscles (IFMs). Postembryonically, these wing disc-associated AMPs undergo rapid rounds of proliferation and subsequent myoblast fusion to form adult fibers, each made up of ~3000 myonuclei. Thus, there is a 1000-fold increase in the size of the myoblast pool within 96 hrs to attain proper muscle size. We have discovered that loss of *moleskin (msk),* which encodes for *Drosophila* Importin 7 (DIM7*)*, in these wing disc-associated myoblasts reduces the overall AMP pool size, resulting in the absence of IFM formation. This myoblast loss is due to a decrease in the AMP proliferative capacity and is independent of cell death. In contrast, disruption of *msk* during pupal myoblast proliferation does not alter the AMP number, suggesting that *msk* is specifically required for larval AMP proliferation. It has been previously been shown that Wingless (Wg) signaling maintains expression of the Vestigal (Vg) transcription factor in proliferating myoblasts. However, other factors that influence Wg-mediated myoblast proliferation are largely unknown. Here we examine the interactions between *msk* and the Wg pathway in regulation of the AMP pool size. We find that reduction of *msk* in the myoblasts results in the absence of Vg expression and a complete loss of the Wg pathway readout β-catenin. Collectively, our results provide strong evidence that *msk* acts through the Wg signaling pathway to control myoblast pool size and muscle formation. We are currently exploring whether DIM7 protein regulates β-catenin stability or nuclear transport, a known function of the Importin protein family.

227B Overactivation of innate immune processes disrupts muscle homeostasis in *Drosophila melanogaster* *Nicole Green*, Justin Walker, Molly Zych, Erika Geisbrecht. Biochemistry & Molecular Biophysics, Kansas State University, Manhattan, KS.

In vertebrates, muscle tissue damage is mitigated by the invasion of immune cells and activation of the inflammatory response. While acute periods of immune activity are reparative, long-term stimulation of the immune response can lead to tissue damage and contribute to muscular dystrophies and myopathies. We are using the *Drosophila* larval muscle attachment site (MAS) to understand the connection between immune activation via damage-associated molecular patterns (DAMPs) and the mechanism by which persistent immune signaling advances muscle pathologies. We have identified a protein called Fondue (Fon) which acts in a novel ECM-driven muscle damage response. Recently, Fon has been characterized as a critical factor for maintaining the *Drosophila* MAS. Both *fon* mutants and *fon* RNAi fillets contain body wall muscles that detach with large gaps between subsets of muscles across hemisegments. Using TEM to analyze MAS ultrastructure, we found that *fon* mutants have disrupted cuticle and tendon architectures, a lack of muscle-tendon interdigitation, and a loss of electron-dense matrix accumulation. These experiments suggest that Fondue is essential for preserving the integrity of the MAS. In addition to muscle phenotypes, loss of *fon* results in excessive melanization at wound sites, spontaneous melanotic nodules in the hemocoel, and constitutive expression of the antimicrobial peptide (AMP), *drosomycin*. These phenotypes are similar to mutants with overactive Toll signaling suggesting that *fon* may interact with Toll pathway members. Furthermore, expression of a constitutively active Toll receptor is sufficient to cause muscle hypercontraction and detachment. Through a fon-sensitized background assay, we find genetic interactions between fon and several members of the Toll pathway such as the NFκB inhibitor, *cactus*. We propose that tissue stresses caused by altered

mechanical forces of weakened and damaged muscle attachment sites leads to overactivation of Toll signaling and the progression of muscle disease.

228C The organization & development of tricellular junctions in *Drosophila* epithelia *T. Matzat*, V. Auld. Zoology, University of British Columbia, Vancouver, British Columbia, Canada.

Epithelial cells have evolved to shield the underlying tissue from diverse and changing environmental influences. They act as paracellular diffusion barrier by forming specialized junctions that preserve tissue integrity. Vertebrate epithelia form tight junctions, whereas invertebrates establish pleated septate junctions (pSJs). An important feature of all epithelia in both vertebrates and invertebrates is the presence of specialized junctions at the point where three cells converge, known as tricellular junctions (TCJs). So far, very little is known about these structures, their organization and their development. However, their presence is necessary for epithelial barrier function but more intriguingly TCJs are also important organizing centers. TCJs modulate the cytoskeletal organization, serve as polarity cues, which are required for the organization of cell geometry and mechanical sensing. Additionally, they play a crucial role during trans-endothelial migration of neutrophils and metastatic cancer cells.

Although the discovery and the initial ultrastructural analysis of TCJs was made around 30 years ago, astonishingly little is known about the molecular components. In *Drosophila*, so far only two TCJ components could be identified: Gliotactin (Gli) and Bark beetle (Bark). A current hypothesis predicts Bark to act as a tricellular-sealing element (“tricellular plug”), whereas Gli might act as a linker between the plugs and the bicellular pSJ strands. We want to test the current hypothesis for *Drosophila*, uncover the exact localization of both proteins and identify their function. To answer these questions, we are using the wing imaginal disc of *Drosophila* as a model system in combination with a set of different electron microscopy techniques.

High resolution TEM tomography in combination with Lanthanum impregnation is used to unravel the connection of the tricellular plugs with the converging septate junction strands in 3D. This method has successfully been used to corroborate the morphological description found in amphipods to *Drosophila*. Furthermore, we are adapting this method for immunolabeling identifying the ultrastructural localization and hence also the function of Gli and Bark at the TCJ. Additionally, we use Focussed Ion Beam SEM to understand and model the overall morphology of TCJs and pSJ in the wing imaginal disc. Current results confirm the proposed model for TCJ junctions, however, challenge the notion of the repetitive organization of the pSJ strands between the epithelial cells leading to the generation of equally spaced septae along all bicellular contacts.

229A Dissecting the interaction between APC2 and ApepP in regulation of Beta-catenin protein levels *Hannah M. Kolev*[1], Samantha L. Smith[1], Tuan A. Thahireen[1], Malachi A. Blundon[1], Scott B. Ferguson[2], Jonathan S. Minden[1], Brooke M. McCartney[1]. 1) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Biology Department, SUNY Fredonia, Fredonia, NY.

The canonical Wnt signaling pathway is evolutionarily conserved from flies to humans, and aids in the regulation of embryonic patterning, tissue maintenance, and metabolic homeostasis. Inappropriate activation of the Wnt signaling pathway leads to a number of disease states including type II diabetes and colorectal cancer. *Adenomatous Polyposis Coli* (*APC*) is a colorectal cancer tumor suppressor and as part of the destruction complex is an essential negative regulator of canonical Wnt signaling. To understand the broad cellular consequences of inappropriate Wnt pathway activation, we compared the proteomes of wild type and APC2 *null* embryos. Through this two-dimensional difference gel electrophoresis (2D-DIGE) proteomic screen, we identified a functional relationship between APC2 and Aminopeptidase P (ApepP) in regulating Beta-catenin (β-cat) protein abundance to prevent inappropriate Wnt pathway activation in *Drosophila* embryos. Metalloaminopeptidases like ApepP remove N-terminal amino acids adjacent to a prolyl residue from proteins and peptides. Interestingly, inappropriate activation of the Wnt pathway in embryos resulted in the accumulation of a post-translational change in ApepP that we predicted could alter its enzymatic activity. Using an *in vitro* assay of ApepP activity we found that in the absence of APC2, ApepP activity is significantly reduced in embryo lysate suggesting that APC2 promotes ApepP’s enzymatic activity. To understand how ApepP activity promotes β-cat destruction, we are using S2 cells to ask if ApepP localizes to and associates with the destruction complex, and we are generating a null allele of ApepP using CRISPR to further dissect the role of ApepP in the negative regulation of Wnt signaling.

230B The Role of Actin-Microtubule Crosslinker Shortstop in Cell Division *Evan Dewey*, Christopher Johnston. Dept of Biology, University of New Mexico, Albuquerque, NM.

Properly executed cell division is crucial to development, maintenance, and longevity of multicellular organisms. Defects in both symmetric and asymmetric divisions can lead to improper developmental patterning, as well as genomic instability, disruption of tissue homeostasis, and cancer. Thus, elucidating the molecular components and their mechanisms of action that regulate these processes has significant importance to human health. Our research focuses on understanding how regulators of the actin and microtubule (MT) cellular cytoskeleton communicate to orchestrate the orientation and stability of the mitotic spindle, a critical component to proper cell division. Shortstop

(Shot) is a member of the spectraplakin protein family found previously to crosslink actin and microtubule filaments, playing a key role in stabilizing interphase microtubules in both *Drosophila* and human cell models. We have found a novel role for Shot in oriented cell divisions, with both tissue culture and *in vivo Drosophila* epithelial models showing spindle misalignment in Shot knockdowns (KDs). Further, we show a role for Shot in spindle assembly in these contexts, demonstrating that spindles do not contain tightly focused poles. Shot KDs also produce defects in chromosomal migration to spindle equator (congression) and chromosomal segregation. We show these activities are likely mediated through traditional Shot roles in stabilization of spindle microtubules (MTs) through crosslinks to actin, but also through a novel, direct interaction of Shot actin binding domain to dynein activator subunit actin-related protein 1 (Arp1) filaments. We hypothesize that this novel interaction with Arp1 functions to crosslink it to spindle MTs, facilitating activation and stabilization of the MT motor protein Dynein, and promoting its activity in spindle assembly, alignment, chromosomal congression, and chromosomal segregation. In support of this model, live cell imaging experiments show defects in cell division timing under Shot KD conditions, and further display defects in chromosomal congression velocities. We find further that Shot loss in epithelial tissue *in vivo* leads to an increase in apoptosis, in line with previous findings linking spindle regulators to cell death. Our research points to important and previously uncharacterized roles of Shot in cell divisions, identifying a new component in a process critical to development and maintenance of multicellular organisms.

231C Function of the Iron-sulfur Cluster Assembly Protein Ciao1 in Growth Regulation in Drosophila Eunbyul Yeom[1,2], *Jean Jung*[1]. 1) Korea Advanced Institute of Science and Technology, Daejeon, South Korea; 2) Korea Research Institute of Bioscience & Biotechnology, Daejeon, South Korea.

Successful growth regulation is vital for proper organ development. Ciao1 is a component of the cytosolic iron-sulfur cluster assembly (CIA) targeting complex along with MMS19 and MIP18/FAM96B that has previously been known for its role in biogenesis of Fe/S proteins including Xeroderma pigemntosum group D (XPD). Studies on Xpd have revealed that this DNA helicase is not only involved in transcription and DNA repair, but it is also essential for coordinating mitosis and genome stability. Here, we show that *Drosophila* Ciao1 has an important role in regulating growth control. Ciao1 shows strong physical interaction with Crumbs, in accordance with its role as a tumor suppressor, and *galla* (*Drosophila* homologue of MIP18), a gene discovered for its importance in chromosome segregation. Reduction of Ciao1 is associated with various organ defects during the developmental stages, and Ciao1 null deletion mutant shows embryonic lethality, thereby placing emphasis on its vital role in development. Ciao1 mutants show decreased levels of Cyclin E and DIAP1, the target genes of the Hippo signaling pathway, the major conserved mechanism for organ growth. Genetic interaction between Ciao1 and Xpd shows that Xpd loss-of-function phenotype can be suppressed by Ciao1 overexpression, and this rescue phenotype is also observed by Cyclin E overexpression. Taken altogether, this study indicates the role of Ciao1 in growth control and, based on the connection to Xpd and Galla, opens possibilities for this iron-sulfur assembly protein in having a functional role in Hippo signaling for growth control.

232A Differential regulation of Cyclin E by Yorkie-Scalloped signaling in organ development *Zhiqiang Shu*, Wu-Min Deng. Department of Biological Science, Florida State University, Tallahassee, FL.

Tissue integrity and homeostasis are accomplished through strict spatial and temporal regulation of cell growth and proliferation during development. Various signaling pathways have emerged as major growth regulators across metazoans; yet, how differential growth within a tissue is spatiotemporally coordinated remains largely unclear. Here, we report a role of a growth modulator Yorkie (Yki), the *Drosophila* homolog of Yes-associated protein (YAP), differentially regulates its targets in *Drosophila* wing imaginal discs, whereby Yki interacts with its transcriptional partner, Scalloped (Sd), the homolog of the TEAD/TEF family transcription factor in mammals, to control an essential cell-cycle regulator Cyclin E (CycE). Interestingly, when Yki was coexpressed with Fizzy-related (Fzr), a *Drosophila* endocycle inducer and homolog of Cdh1 in mammals, surrounding hinge cells displayed larger nuclear size than distal pouch cells. The observed size difference is attributable to differential regulation of CycE, a target of Yki and Sd, the latter of which can directly bind to *CycE* regulatory sequences, and is expressed only in the pouch region of the wing disc starting from the late second-instar larval stage. During earlier stages of larval development, when Sd expression was not detected in the wing disc, coexpression of Fzr and Yki did not cause size differences between cells along the proximal-distal axis of the disc. We show that ectopic CycE promoted cell proliferation and apoptosis, and inhibited transcriptional activity of Yki targets. These findings suggest that spatiotemporal expression of transcription factor Sd induces differential growth regulation by Yki during wing disc development, highlighting coordination between Yki and CycE to control growth and maintain homeostasis.

233B Genetic Control of Tissue-Specific Growth in the Larval Trachea of *Drosophila* *Kayla Wilson*, Latavia Hill, Erin Suderman, Robert Ward. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

In humans and many animals, post-embryonic development is achieved through allometric growth. Allometric growth is characterized by organs and tissues that grow at different rates relative to each other. In other words,

Allometric growth is tissue-specific, and while it is known that the growth of each organ or tissue is dependent on its function in development and homeostasis, the mechanisms that control this growth are poorly understood. In order to elucidate tissue-specific growth mechanisms, we are using the larval trachea in *Drosophila melanogaster* as a model tissue. The trachea is the gas exchange organ in *Drosophila.* Larval tracheal growth is an excellent model to study tissue-specific growth because the trachea can be easily imaged and measured in live animals, it is composed of a tubular epithelial network that exhibits tissue-specific growth, and various genetic approaches can be employed to manipulate gene expression specifically in the trachea. The genes *uninflatable* (*uif*) and *Matrix metalloproteinase 1* (*Mmp1*) have been identified as tissue-specific growth regulators of the larval trachea. Larva with homozygous mutations in these genes have trachea that are about half the relative size of those found in wild type animals. To identify additional genes involved in larval trachea growth, we screened through a collection of EMS-induced larval lethal mutations, and identified 7 mutants that have an abnormal ratio of trachea to body length in 3rd larval instars. Three of the mutants show reduced tracheal growth similar to mutations in *uif* and *Mmp1*, whereas the remaining 4 mutant lines show increased tracheal growth. Three of these are allelic, and we have used deficiency mapping, complementation analysis and RNAi to determine that these three alleles are mutations in *CG11340*. The fourth overgrown tracheal mutant (*l(3)LL12265*) fails to complement *Df(3R)bsc547*, a deficiency that spans 99B5 to 99C2 and includes 50 genes. One of the reduced tracheal growth mutant lines (*l(3)LL15149*) fails to complement a pair of overlapping deficiency that span 96B15 to 96C3 and includes 40 genes. We are currently conducting complementation analysis using overlapping deficiencies, and RNAi against candidate genes in order to identify the specific genes responsible for these two mutants. In addition, we are examining the cellular phenotypes associated with all of these mutations in order to uncover molecular mechanisms associated with tracheal-specific growth that can be used as a paradigm to better understand allometric growth in all organisms.

234C Spindle Orientation: How Complex is the Complex? Nicole Dawney, Tyler Wilson, *Dan Bergstralh*. Department of Biology, University of Rochester, Rochester, NY.

Precise control of division orientation is important to a variety of developmental processes, ranging from cell fate asymmetry to epithelial tissue expansion. The direction of cell division is determined by the orientation of the mitotic spindle at metaphase, and for this reason spindle orientation has been well studied across a range of model systems. In worms, flies, and vertebrates, spindle orientation relies on Mud and Dynein, which combine to exert a pulling force on astral microtubules. In nearly every mitotic cell type examined, these proteins are anchored to the cortex by Gαi and Pins, which are thought to work in combination with a growing number of putative accessory factors. However, recent work reveals the unexpected finding that spindle orientation in the Drosophila imaginal wing disc is Pins-independent. This raises the possibility that spindle orientation is more diverse, and not as well understood, as previously thought. Here we present ongoing work aimed at understanding the spindle orientation machinery in Drosophila epithelial tissues.

235A Break-induced replication in the *Drosophila* germline *Travis Karg*[1], Jayaram Bhandari[1], Mitch McVey[2], Kent Golic[1]. 1) University of Utah, Salt Lake City, UT; 2) Tufts University, Boston, MA.

Chromosomes with a double stranded DNA break (DSB) can be efficiently repaired by several mechanisms that rejoin the two broken ends. However, these repair mechanisms are inadequate to repair a single broken end in the absence of a second broken end to which it may join. A broken end that is left unrepaired can lead to genomic instability, which is considered a hallmark of cancer. But in most cases an unrepaired break directs that cell to be destroyed by apoptosis. Occasionally a broken end may be recapped by the addition of a new telomere in a process called healing. However, this event may lead to loss of genetic information from the end of the chromosome.

In yeast, a broken chromosome can also be restored through a process called “Break-Induced Replication” (BIR). The BIR machinery can use a sister chromatid or homologous chromosome as a template and initiate DNA replication to fully restore chromosome length. Extensive BIR has not been reported in higher eukaryotes, though it bears similarity to the ALT (Alternative Lengthening of Telomeres) mechanisms of telomere maintenance utilized by some cancer cells.

We tested whether the homologous chromosome could be used as a template to repair chromosomes by BIR in Drosophila. By inducing recombination between two inverted FRT sites on sister chromosomes, we efficiently induce the formation of a dicentric chromosome. During mitosis, the dicentric is broken, leaving two daughter cells with a single broken chromosome end. We find that BIR can be used to repair the broken chromosome, and that the BIR repair tract may proceed as far as 1 Mbase. Although BIR is several-fold less efficient than healing, it can account for as much as 10% of the repaired chromosomes recovered through the germline. We are currently examining the effects of mutants that are known to affect BIR in yeast to determine whether the mechanism of BIR is conserved.

236B Investigating the Mcm10/RecQ4 genetic interaction in Drosophila Melanogaster *Christopher Knuckles*, Tim Christensen. Biology, East Carolina University, Greenville, NC.

Necessary to the survival of cellular life is proper replication and maintenance of the genome. Replication proteins Mcm10 and RecQ4 have well-characterized essential roles in assembly, initiation, and proper functioning of the eukaryotic replication machinery. Mcm10 enables efficient assembly of the replication fork while RecQ4 delivers the GINS complex; both of which are pivotal for origin firing. Recent studies suggest that Mcm10 is not required for RecQ4 chromatin localization or association with the CMG complex, conflicting with a previous report that Mcm10 mediates the interaction between RecQ4 and Mcm2-7 in an S-CDK dependent manner. We have found that a homozygous mutation of RecQ4 is lethal in fruit flies unless they possess a C-terminal domain (CTD) truncation of Mcm10, suggesting that Mcm10 and RecQ4 display a genetic interaction. Here, we investigate how an Mcm10/RecQ4 genetic interaction might be important to facilitate high-stress replication states – like oogenesis endoreplication and larval brain development – and less so for normal cell cycles. The Mcm10 CTD truncation rescue of lethal RecQ4 phenotypes can reveal much about how the cooperative roles of these proteins affect DNA replication, but this requires further investigation. Our research aims to explore the nature of the Mcm10/RecQ4 genetic interaction by conducting genetic investigations, proliferation assays, and double strand break (DSB) studies in the *Drosophila* ovary and larval brain. We propose that Mcm10 and RecQ4 genetically interact to facilitate DNA replication in *Drosophila melanogaster*.

237C FLYINGLOW: biological effects of protracted low radiation doses in *Drosophila melanogaster* F. Cipressa[1,2], P. Morciano[1], G. Esposito[2,3], A. Porrazzo[1], D. Iovino[4], R. Iorio[4], L. Satta[2], M.A. Tabocchini[2,3], *G. Cenci*[1,2]. 1) SAPIENZA University of Rome, Rome, Italy; 2) Centro Studi e Ricerche 'Enrico Fermi', Rome, Italy; 3) Istituto Superiore di Sanità (ISS) and INFN-Gr.coll.Sanità, Rome, Italy; 4) Università of L'Aquila, L'Aquila, Italy.

Natural background radiation of Earth and cosmic rays played a fundamental role during the evolution of living organisms. However how low doses of irradiation can affect biological processes is still unclear. We have previously shown that cells grown at the Gran Sasso Underground Laboratory (LNGS) of INFN, in which dose rate of ionizing radiations and neutrons is significantly reduced with respect to the external laboratories, elicited an impaired response against endogenous damage as compared to cells grown outside LNGS. This suggests that environmental radiation contributes to the development of defence mechanisms at cellular level. To further understand how environmental radiation affects metabolism, we have recently launched the FLYNGLOW program that aims at exploiting *Drosophila melanogaster* as a model for evaluating the effects of low doses of irradiation at organismal level. We will present a comparative data set on lifespan, fertility and response to genotoxic stress from different Drosophila strains grown in parallel at LNGS and in reference laboratories at different levels of gamma radiation background.

238A Highways for repair: nuclear actin and myosin drive the relocalization of heterochromatic DNA damages to the nuclear periphery during homologous recombination. Carla D'Agostino, Taeyun Ryu, Xiao Li, Nuno Amaral, Harianto Tjong, Frank Alber, *Irene Chiolo.* Los Angeles, CA.

Heterochromatin mostly comprises repeated sequences prone to harmful ectopic recombination during double strand break (DSB) repair. In Drosophila cells, 'safe' homologous recombination (HR) repair of heterochromatic breaks relies on a specialized pathway that relocalizes damaged sequences away from the heterochromatin domain before strand invasion. However, the mechanism responsible for this movement was a mystery. Here we show that, strikingly, the relocalization of heterochromatic DSBs occurs with directed motions. These depend on specific nuclear myosins that are recruited to heterochromatic DSBs at early stages of repair, and on and the 'ad hoc' formation of nuclear actin filaments by actin nucleators. These activities and nuclear structures work in concert with Smc5/6 for driving the relocalization of heterochromatic DSBs to the nuclear periphery and completing repair in a 'safe' environment. Losing these motor functions results in massive chromosome rearrangements and genome instability, revealing the importance of these 'highways for repair' in heterochromatin integrity. By revealing an unexpected role of nuclear motors in the spatial and temporal regulation of HR repair in heterochromatin, this study uncovered the importance of nuclear actin and myosin components in genome stability in multicellular eukaryotes.

239B Impact of chromatin modifications on heterochromatic double strand break repair *Laetitia Delabaere*[1], Helen Chung[1], Gaelle Legube[2], Irene Chiolo[1]. 1) Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2) CBI, Universite Paul Sabatier, Toulouse, France.

The abundance of repeated sequences in heterochromatin renders double strand break (DSB) repair a major challenge due to the risk of aberrant recombination. Using the *Drosophila* Kc cells, we have recently identified a unique pathway that regulates homologous recombination (HR) repair in space and time to prevent aberrant recombination between heterochromatic sequences. Repair starts inside the heterochromatin domain where HR progression is blocked after DSB resection. Next, repair sites relocalize to the nuclear periphery to resume HR repair

in a 'safe' environment. Previous studies in euchromatin revealed that DNA damage signaling and repair rely on specialized histone modifications. However how the unique 'silent' chromatin environment contributes to the dynamic DSB response in heterochromatin is unknown. What chromatin changes occur at heterochromatic DSBs and their roles in repair progression are also poorly understood. To address these questions, we optimized new tools to generate site-specific DSBs in the *Drosophila* genome by expressing the rare-cutting AsiSI restriction enzyme in Kc cells. Combining imaging techniques and chromatin-immunoprecipitation (ChIP) experiments, we showed that this tool allows a rapid induction of DSBs in both euchromatin and heterochromatin. Importantly, AsiSI-induced heterochromatic DSBs relocalize to outside the domain and are repaired within a few hours, similar to what previously observed in response to ionizing radiation. Using this powerful tool, we have investigated histone modifications occurring specifically at heterochromatic DSB by ChIP. We have also investigated the chromatin modifiers responsible for these modifications and their role in repair. We will present our most recent discoveries on this pathway. Aberrant recombination contributes to aging, developmental defects and cancer. Thus, identifying the mechanisms required for proper heterochromatic DSBs are expected to generate a better understanding of mutations responsible for human diseases, and novel perspectives for their prevention and cure.

240C Utilization of transient secondary-structure forming sequences during alternative end joining repair of double-strand breaks *Terrence Hanscom*[1], Varandt Khodaverdian[1], Steven Roberts[2], Mitch McVey[1]. 1) Department of Biology, Tufts University, Medford, MA; 2) School of Molecular Biosciences, Washington State University, Pullman, WA.

End-joining repair of DNA double-strand breaks can occur via Ku and Ligase 4-dependent processes (classical NHEJ) or via Ligase 4-independent processes. The latter repair is termed alternative end joining (alt-EJ) and is highly mutagenic, producing deletions, insertions, and chromosome translocations. We have evidence that both insertional events and junctions involving short microhomologies involve nascent DNA synthesis, and we hypothesize that the cells are using single-stranded DNA adjacent to the break site to form secondary structures that are used as primers for synthesis. The nascent DNA is then unwound and used to anneal broken DNA ends. Genetic evidence suggests that the synthesis is catalyzed by the Drosophila orthologue of translesion DNA polymerase theta (encoded by *mus308*). We have proposed a model, termed synthesis-dependent microhomology-mediated end joining (SD-MMEJ), to explain how seemingly different types of alt-EJ products can be formed through a common mechanism and are currently elucidating the "rules" that govern SD-MMEJ in Drosophila.

Here we report the use of a novel *in vivo* DNA repair assay to characterize alt-EJ. The assay uses plasmid constructs containing an *I-SceI* endonuclease cut site with subtle nucleotide changes in the area surrounding the cut. The sequence changes were designed to elicit differences in repair outcomes due to the presence or absence of secondary structure-forming sequences. Plasmids were injected into Drosophila embryos lacking DNA ligase 4 and expressing *I-SceI* endonuclease and incubated for four hours. The repaired plasmids were then recovered, purified, and next-generation amplicon sequencing was performed. Preliminary results suggest that alt-EJ repair commonly utilizes DNA hairpins and loops as primers, but that these structure-forming sequences must be of optimal length to assemble and disassemble rapidly. Our data supports the SD-MMEJ model and can explain the presence of insertions, deletions, and blunt joins in alt-EJ repair products. We are now characterizing the relationship between sequence context, secondary structure, and polymerase theta protein activity which will have a great impact on fields such as genomic engineering and editing using tools such as CRISPR-Cas9.

241A Dicentric Ring-X chromosomes have clustered breakpoints indicating fragile regions. *Hunter J Hill*, Kent Golic. Biology, University of Utah, Salt Lake City, UT.

Chromosomal constrictions were first observed by Calvin Bridges in Drosophila salivary glands about a century ago. These sites replicate later than the rest of euchromatin, resulting in under replication in endocycling cells. In 1939 B.P. Kauffmann found that these regions of euchromatin acted very much like centromeric heterochromatin and appropriately called them intercalary heterochromatin (IH). Importantly, the IH constrictions also replicate late in mitotic cells and resemble the constrictions that exist in human chromosome 'fragile sites'. Charles Laird and colleagues proposed that IH constrictions and fragile sites in human chromosomes are analogous. We aim to further understand chromosome fragility and determine if indeed chromosomes are fragile in flies and humans for the same reasons.To address the notion that chromosomes could break in preferred locations, we devised a screen to recover broken-and-healed dicentric chromosomes. Utilizing an FRT bearing Ring-X chromosome, and nanosGal4 paired with UAS FLP we induced a single exchange between ring sister chromatids in the male germline. The resulting double bridge carries two identical copies of the X chromosome, and can theoretically break anywhere. Our screen was designed to recover broken and then healed chromosomes in viable and fertile males, restricting the possible breakpoint locations to those generating a near-euploid male i.e. chromosomes where the breakpoints in each arm of the bridge are near each other. We predicted most recovered chromosomes would have breaks in heterochromatin because there are no essential genes in X heterochromatin and the breakpoints could be staggered by large distances. Surprisingly we found half of the new termini in euchromatin, generating a collection of metacentric X

chromosomes. Furthermore, the euchromatic breaks are in clusters surrounding regions previously identified as intercalary heterochromatin. We are interested in the source of fragility at these locations, and specifically, we wish to identify the precise DNA sequences at the breakpoints. Chip-seq of telomere proteins on the newly healed ends will determine the breakpoints with base-pair resolution. Identification of these sequences is not only an essential step in shaping the analogy between IH and fragile sites, it will also provide insight on the process of chromosome breakage and healing by allowing us to determine if there are any specific sequences or chromatin features that correlate with the common sites of breakage.

242B The role of DmBlm in repair of simple DNA double-strand breaks *Jeannine LaRocque*, Henry Ertl, Daniel Russo, Noori Srivastava, Thu Dao. Human Science, Georgetown University, Washington, DC.

The maintenance of genome integrity is necessary to ensure accurate transmission of genetic information to the next generation. DNA double strand breaks (DSBs) are a particularly deleterious class of DNA damage that threatens the integrity of the genome. DSBs are repaired by three pathways: non-homologous end joining (NHEJ), single-strand annealing (SSA), and homologous recombination (HR). *Drosophila melanogaster* Blm (DmBlm; encoded by *mus309*) is the ortholog of yeast RecQ helicase *sgs1* and human *Blm* and has been shown to suppress crossovers in mitotic cells and repair mitotic DNA gaps via HR. To determine the role of DmBlm in repair of a simple DSB by NHEJ, HR, or SSA, the DR-*white* and DR-*white.mu* DSB repair assays were utilized. DmBlm null mutants demonstrated a decrease in repair by HR, a concurrent increase in SSA and crossovers, and an increase in NHEJ events, although processing of the ends is not significantly impacted. Interestingly, gene conversion tract lengths associated with HR were substantially shorter in DmBlm null mutants compared to heterozygote controls. Using DR-*white.mu*, we found that in contrast to in yeast Sgs1, DmBlm is not required for suppression of recombination between diverged sequences. Our data suggests that DmBLM plays a role in repairing a simple DSB by HR, thus impacting DSB repair pathway choice, and provides more insight on how this protein functions to maintain genome integrity.

243C Cell cycle re-entry in the optic lobes of the adult *Drosophila* brain *Shyama Nandakumar*, Olga Grushko, Laura Buttitta. Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

In recent years, it has been established that neuronal aneuploidy is more common than previously thought, and prevalent in many organisms. Aberrant cell cycle events and increased ploidy in postmitotic neurons have also been associated with neurodegenerative states. How and where is aneuploidy generated, and what role does it play in neurodegeneration? We find that under normal ageing conditions, cells in aged *Drosophila melanogaster* brains exhibit increased hyperploidy. We developed a sensitive flow cytometry based assay to quantify this phenomenon on a single brain scale to uncover rare and possibly transient changes in DNA content. In addition, we can simultaneously monitor cell death and examine different subpopulations of cells using the *Drosophila* transgenic GAL4/UAS system to label specific cell types. We find that both neurons and glia exhibit increased hyperploidy in aged brains, and that the optic lobes contribute to the majority of hyperploid cells. We also observe a constant rate of cell death in the ageing brain, suggesting the ageing brain suffers a continual loss of cells. Other adult postmitotic tissues in *Drosophila* have been shown to undergo cell cycle reentry to compensate for cell loss, and we suggest compensatory cell cycle reentry may also occur in the aged adult brain. Our preliminary data suggests specific subsets of neurons in the optic lobes are more susceptible to hyperploidy and cell cycle reentry. We are therefore employing behavioural vision assays to determine the biological significance of the hyperploidy and cell cycle reentry in the optic lobes. We can also manipulate the cell cycle machinery with spatio-temporal specificity in the brain, and using these tools we have demonstrated that forced cell cycle reentry in postmitotic neurons and glia leads to neurodegeneration.

244A Role of Polyploid Glial Cells in the Peripheral Nervous System *Laura E. Frawley*[1,2], Terry L. Orr-Weaver[1,2]. 1) Whitehead Institute for Biomedical Research, Cambridge, MA; 2) Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Development of an organ relies on the coordinated growth among different cell types within given tissues. The massive organismal growth observed between the three larval stages is a consequence of increased cell size from increased ploidy rather than increased cell number. Our lab has demonstrated that polyploidy is utilized to control glia cell size in the nervous system (Unhavaithaya et al., 2012). We have shown that two glia cell types are polyploid, subperineurial glia (SPG) and wrapping glia (WG). Polyploid SPG are required to maintain the integrity of the blood-brain barrier, whereas polyploid WG ensheath axons in the peripheral nervous system (PNS) (Unhavaithaya et al., 2012 and Stork et al., 2008). In the PNS, lineage tracing analysis showed that there are three WG and four SPG per nerve, which are all post-mitotic during larval development (von Hilchen et al., 2013). An important question is how in the absence of increased cell number these cells can compensate for a 10-fold increase in nerve length between the shortest and longest nerve during one larval instar as well as up to a 50-fold increase in length of each nerve during larval development. We determined that a strong correlation between nerve length and total WG ploidy exists within a given animal. We are investigating the function of polyploidization in WG, the relationship between SPG and WG

ploidy, and the consequence of regulating cell size rather than cell number.

Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, Klambt C. (2008). Organization and function of the blood–brain barrier in Drosophila. *J Neurosci* 28: 587–597.

Unhavaithaya, Y., & Orr-Weaver, T. L. (2012). Polyploidization of glia in neural development links tissue growth to blood-brain barrier integrity. *Genes & Development* 26, 31–36.

von Hilchen, C.M., Bustos, A.V., Giangrande, A., Technau, G.M., Altenhein B. (2013). Predetermined embryonic glial cells form the distinct glial sheaths of the Drosophila peripheral nervous system. *Development* 140, 3657-3668.

245B Variant cell cycles ensure a functional blood-brain barrier in Drosophila *Jessica R. Von Stetina*[1], Laura E. Frawley[1], Yingdee Unhavaithaya[2], Terry L. Orr-Weaver[1]. 1) Depart. of Biology, Whitehead Institute for Biomedical Research, Cambridge, MA; 2) Citeline, 52 Vanderbilt Ave., 11th Floor New York, NY.

Developmental programmed polyploidy is used in both plants and animals as a strategy to increase cell size. In Drosophila, the growth of most differentiated larval and adult tissues is driven by increases in DNA content rather than by increases in cell number. Polyploidy can arise from two cell cycle variants, the endocycle and endomitosis. During the endocycle, alternating cycles of DNA replication and growth take place in the absence of mitosis, yielding cells with a single, polyploid nucleus. In one form of endomitosis, most aspects of mitosis including nuclear division occur in the absence of cytokinesis, resulting in polyploid multinucleated cells. Our previous studies demonstrated that the subperineurial glia (SPG), which form the blood-brain barrier (BBB), grow by increasing ploidy without an increase in cell number. This strategy permits the SPG to increase in size to accomodate increased underlying neuronal mass to maintain the integrity of the BBB, without disruption of septate junctions by cell division and cytokinesis (1). We also showed that SPG are unique in polyploidizing by both the endocycle and endomitosis (1). Here we find that all the SPG initially transition into the endocycle during embryogenesis, then the majority of the SPG in the brain lobes switch to endomitosis during larval development. The decision to transition from the endocycle to endomitosis requires the mitotic activator Cdc25 phosphatase (String) and is inhibited by Notch signaling. Reduction of Notch pathway components or alterations in String activity alter the endocycle to endomitosis ratio in SPG and lead to defects in the BBB. Our results reveal that increased nuclear number increases cell size, as even at the same ploidy, endomitotic SPG are larger than endocycling SPG. In addition, the endomitotic SPG attain higher ploidies. Together our findings suggest that 1) the BBB requires the correct ratio of endocycling and endomitotic SPG and 2) SPG in the brain exploit endomitosis to boost their cell size above the attainable by the endocycle in response to substantial brain growth during larval development.

1. Unhavaithaya, Y. and T. L. Orr-Weaver (2012). Polyploidization of glia in neural development links tissue growth to blood-brain barrier integrity. Genes & Development **26**: 31-36.

246C *Jim lovell* (*lov*) is a Regulator of Larval Endopolyploid Growth *Fanli Zhou*, Karen Qiang, Rami Dibbs, Kathleen Beckingham. Biosciences department, Rice university, Houston, TX.

jim lovell (*lov*) encodes a putative transcription factor of the BTB/POZ domain family. The first mutation to the gene was identified in a screen for behavioral mutants with altered gravitational responses. Immunolocalization studies indicated that *lov* influences behavior through its expression in the nervous system with *lov* first activated in neuronal subsets of the developing larval CNS and PNS. Working with the Gal4-UAS system to knock *lov* expression down in subclasses of larval neurons, an incompletely characterized *cut*-Gal4 driver was used that induced hypoxia-like behavior in the affected larvae. Subsequent studies established that in the larval stage, this driver is expressed exclusively in the tracheae with very strong expression in the two most posterior sections of the dorsal tracheal trunks. This discovery led to the recognition that *lov* is also expressed in the tracheae throughout the larval stages and that loss of *lov* function in the tracheae results in inhibition of tracheal endopolyploid growth leading to tracheal breakage and fluid filling, followed by hypoxia and hypoxia-induced behavioral responses. Interestingly, *lov* over-expression produces even greater inhibition of tracheal growth. We have determined that *lov* is expressed in several other endopolyploid tissues and that *lov* knockdown in the epidermis and the salivary glands also inhibits endopolyploid growth. As in the tracheae, *lov* overexpression produces stronger growth inhibition than *lov* underexpression in these tissues. In the salivary gland *lov* overexpression also alters the overall morphology of the organ. dMyc and the Wingless and Notch pathways are the best characterized regulators of endopolyploidy in Drosophila. Lov, like dMyc, has proved to be a nucleolar protein. Studies to determine the molecular action of Lov in endopolyploid growth in relation to these known regulators are in progress.

247A Investigating the role of inflammatory cytokines on tumor progression and metastasis in a *Drosophila* cancer model *Kirti Snigdha*[1], 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 (TREND), University of Dayton, Dayton OH; 3) Premedical Programs, University of Dayton, Dayton OH.

Tumor cells and surrounding normal cells interact among each other and constitute the tumor microenvironment (TME). The TME supports the survival and proliferation of tumors. Studies indicate the presence of inflammatory

components in the TME. However, little is known about the effect of these inflammatory molecules on tumor growth and progression, and on the surrounding stromal (normal) cells. The core inflammatory pathways e.g. TLR, TNF etc. are conserved in *Drosophila melanogaster*. Imaginal discs of *Drosophila melanogaster* are a popular model to study epithelial tumors due to the variety of mosaic analysis tools, and the ease positively marking cells that allows tracking growth and progression of tumor cells. We co-activated oncogenic forms of *Yki* or Ras^{V12} activities in polarity deficient (*scribble* mutant) epithelial cells to model aggressively growing and metastatic tumors. To simulate the TME, 'FLP-out' clones of tumor cells marked with GFP were generated. The activity of key inflammatory pathways Toll, TNF and JNK in the TME was tested by immunohistochemistry. We observed upregulation of Cactus (TLR pathway component) and p- JNK (activated form of JNK), and downregulation of *Drosophila* TNF ligand, Eiger in the tumor cells. Of these, only Eiger was upregulated in neighboring normal cells. Based on these results we hypothesized that a bidirectional cross-talk between the tumor and normal cells in the TME elicits differential inflammatory response that promotes tumor cell survival and progression. To analyze this, we down-regulated Cactus and Wengen (*Drosophila* TNF receptor) and evaluated the effect on tumor size and survival of tumor cells. A comparison of hyperplastic (*Yki* $Scrib^{RNAi}$) and metastatic (Ras^{V12} $Scrib^{RNAi}$) tumor model was done to identify the role of inflammatory cytokines in tumor progression. Here, we report our progress on the study of the effect of these pathways and inflammatory cytokines on tumor survival and metastasis. Our research will help unravel the correlation between inflammatory pathways and tumor progression in an *in vivo* model.

248B The Hippo Pathway Acts as a Gatekeeper to Restrict EGFR/Ras Driven Tumorigenesis Justine Pascual[1], Jelle Jacobs[2,3], Leticia Sansores[2], Malini Natarayan[4], Julia Zeitlinger[4,5], Stein Aerts[3], Georg Halder[2], *Fisun Hamaratoglu*[1]. 1) CIG, University of Lausanne, Lausanne, Switzerland; 2) VIB Center for the Biology of Disease and KU Leuven Center for Human Genetics, University of Leuven, Leuven 3000, Belgium; 3) Laboratory of Computational Biology, Center for Human Genetics, University of Leuven, Leuven 3000, Belgium; 4) Stowers Institute for Medical Research, Kansas City, MO, 64110, USA; 5) Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, 66160, USA.

Transformation of a healthy cell into a cancerous one requires multiple mutations. These mutations often influence the activity of genes that control proliferation, cell death, polarity and differentiation during normal development. A handful of signaling pathways such as Wingless/Wnt, BMP/TGFb, Hedgehog, Notch, EGFR and Hippo orchestrate development of all multicellular organisms. How the activity of these pathways are coordinated with each other during development and how the cancer cells overcome this regulation is not well understood. Here, we revisited the interaction between EGFR/Ras signaling and the Hippo pathway. We found that the mutations in EGFR and Hippo pathways interact synergistically to cause excessive growth of *Drosophila* imaginal discs. Interestingly however, although EGFR and Hippo signaling can affect each other's signaling activities, the synergistic interaction between the two pathways did not depend on cross-regulatory activities of the signal transduction cascades but involved an interaction at the level of the downstream transcription factors. We found that loss of Capicua (Cic), a transcriptional repressor that mediates the growth effects of EGFR signaling in imaginal discs, synergistically enhances Yorkie driven overgrowth due to the upregulation of a select set of target genes. Surprisingly, the effect is not due to hyperactivation of known Yki driven growth regulators, but due to ectopic activation of genes that are normally shut down by Cic. We characterize the transcriptional network that leads to this impressive synergy in driving excessive growth.

249C A non-apoptotic function of the caspase Ice regulates Drosophila tracheal length downstream of Hippo signaling *S. McSharry*, B. Kraft, G. J. Beitel. Molecular Biosciences, Northwestern University, Evanston, IL.

cause tracheal size phenotypes that are the opposite of the same mutations in other tissues. For example, a loss-of-function mutation in Yorkie, the transcription factor regulated by the Hippo Network, yields decreased eye/ wing size; this same mutation, however, causes an increase in tracheal elongation, without changing cell number. Overelongation of the trachea in Yorkie mutants is caused by derepression of the executioner caspase Drice, the fly homologue of the mammalian caspase-3. To determine how Drice mediates tracheal elongation without triggering apoptosis, we tested whether tracheal cells were resistant to apoptosis, and whether induction of apoptotic activity was sufficient to increase tracheal length. Overexpression of the pro-apoptotic genes Grim and Reaper in the trachea using the UAS-Gal4 system resulted in Drice-dependent tracheal cell death, with phenotypes ranging from branch discontinuities to near absence of the tracheal system. More limited overexpression of Hid using a heat-shock inducible promoter, however, caused much less cell death and could produce tube-length increases without leading to missing segments. Thus, tracheal cells are not resistant to apoptosis, and tracheal elongation appears to result from a sub-threshold activation of Drice. Staining for active (cleaved) Drice reveals more concentrated staining at the apical surface of the tube, suggesting that compartmentalization of Drice may spatially isolate tracheal length activity from apoptotic activity. To investigate the interplay between previously identified tracheal size-control pathways and the Hippo Network, we are using classic epistasis experiments as well as a modified approach in which we have

reengineered DIAP1 to be expressed exclusively under the control of a constitutive promoter, removing it from Yorkie-mediated regulation. By making double mutant combinations of this constitutive DIAP locus with other tracheal mutations, we will be able to determine which genes act through Yorkie-regulated Ice activity to control tracheal length.

250A Warts signaling coordinates organ growth with body size through regulation of ecdysone Morten E. Moeller, Stanislav Nagy, Stephan Gerlach, Karen Soegaard, E. Thomas Danielsen, *Kim Rewitz*. Biology, University of Copenhagen, Copenhagen, Denmark.

During development, animals must coordinate body growth and the growth of individual organs with developmental transitions to produces an adult with correct size and proportions. In *Drosophila*, growth and development of organs are controlled by integration of systemic signals with organ-intrinsic signals. The Warts-Hippo signaling pathway plays a role in an intrinsic size-control mechanism that regulates imaginal disc size. The mechanisms by which local organ-intrinsic signals are integrated with systemic signals to coordinate growth between organs and the whole body have remained poorly defined. We show that during the growth period Warts (Wts) activity, through regulation of ecdysone production, couples nutrition and developmental signals to systemic growth.

Our analysis of wts function in the ecdysone-producing prothoracic gland (PG) indicates that the Wts-Yorkie pathway is a key regulator of ecdysone production that controls systemic growth and is required for growth-coordination to synchronize growth between organs and the body. In the PG, Wts mediate effect of insulin and PTTH on the regulation ecdysone production to adjust the rate of growth in response to environmental conditions. Surprisingly, the activity of Wts in the PG is required for normal growth of the disc tissue. Our data show that Wts plays a key role in regulating ecdysone production required for growth coordination and control of organismal size.

251B Maintenance of tissue homeostasis by mechanical stress sensing *Kenta Morimoto*[1], Emiko Suzuki[1], Wu-Min Deng[2], Yoichiro Tamori[1]. 1) National Institute of Genetics, Mishima, Japan; 2) Florida State University, Tallahassee, FL.

In multicellular organisms, tissue integrity and organ size are maintained through removal of aberrant or damaged cells and compensatory proliferation. The sensing and removal of aberrant cells by their neighbors involve cell competition, a remarkable homeostatic process at the cellular level. In proliferating tissues, cell division is the primary strategy winner cells use to compensate for the loss of loser cells during cell competition. In post-mitotic *Drosophila* follicular epithelia, however, we have shown that the loss of local tissue volume resulting from loser-cells elimination triggers sporadic cellular hypertrophy to repair the tissue. This ‘‘compensatory cellular hypertrophy’’ (CCH) is implemented by polyploidization through the endocycle, a variant cell cycle composed of DNA synthesis and gap phases without mitosis, dependent on activation of the insulin/IGF-like signaling (IIS) pathway. Furthermore, several lines of evidence in our study suggest that the IIS-dependent CCH is triggered by tensile forces resulting from the elimination of loser cells. To identify the genes involved in the mechanotransduction, we used the stretched follicle cells in which cellular stretch-induced extra endocycle and endogenous activation of the IIS are observed. We found that a transient receptor potential (TRP) channel is involved in the endogenous CCH in the stretched follicle cells. Furthermore, a live-cell calcium imaging analysis by using calcium indicator GCamp revealed that intercellular calcium level is endogenously higher in the stretched follicle cells than the columnar main body follicle cells. These data indicate that TRP channel activation in response to mechanical stretching stress induces calcium incorporation, thereby activating IIS pathway. Given the fact that similar homeostatic cellular hypertrophy can be observed in different contexts such as *Drosophila* epidermis, mammalian hepatocytes or corneal endothelial cells, the mechanotransduction-induced CCH is likely a conserved strategy for postmitotic tissue homeostasis.

252C *Drosophila* imaginal disc growth factor 2 is involved in energy balance, detoxification, and defense *M. Zurovec*, V. Broz, L. Kucerova, L. Rouhova. Dept Genetics, Biology Centre CAS, Inst Entomology, Ceske Budejovic, Czech Republic.

Drosophila imaginal disc growth factor 2 (IDGF2) is a member of chitinase-like protein family (CLPs) able to induce the proliferation of imaginal disc cells *in vitro*. We characterized physiological concentrations and expression pattern of IDGF2 *in vivo* as well as its impact on the viability and transcriptional profile of *Drosophila* cells *in vitro*. We showed that IDGF2 supports the growth of Cl.8+ cells independently of insulin in a dose-dependent manner. We also show that IDGF2 protects cells from death caused by serum deprivation, toxicity of xenobiotics or high concentrations of extracellular adenosine and deoxyadenosine to a similar extent as vertebrate serum. In addition, the transcriptional profiles of IDGF2 expression *in vivo*, its localization at garland and pericardial nephrocytes and induction by injury in larval stages support the IDGF2 role in organismal defense and detoxification. Taken together our findings provide evidence that IDGF2 is an important trophic factor promoting cellular and organismal survival.

253A IAP-antagonist expression is not sufficient to induce caspase activation during *Drosophila* endogenous cell death *Sarah Neuman*[1], Yunsik Kang[2], Robert Ihry[3], Arash Bashirullah[1]. 1)

University of Wisconsin-Madison, Madison, WI; 2) Vollum Institute, Oregon Health Science University, Portland, OR; 3) Novartis Institutes for BioMedical Research, Cambridge, MA.

Proper regulation of programmed cell death is critical for both normal development and disease in all animals. In *Drosophila*, initiation of endogenously-regulated programmed cell death is thought to hinge on transcriptional induction of IAP-antagonists like *reaper* and *hid.* However, we have found that IAP-antagonist expression is not sufficient to trigger widespread caspase activation during the destruction of the *Drosophila* larval salivary glands; instead, this response requires coordinate upregulation of both IAP-antagonists and downstream caspases. We find that expression of critical effectors of caspase activation, including the initiator caspase *dronc* and the effector caspase *dcp-1*, is dramatically upregulated in response to the same pulse of the steroid hormone ecdysone that induces expression of *reaper* and *hid* in the salivary glands. Importantly, failure to coordinately increase expression levels of caspases during the death response disrupts the ability of IAP-antagonists to trigger massive caspase activation. Furthermore, our results indicate that these death genes are induced in a carefully orchestrated sequence, beginning with the caspase adapter *dark*, followed by the initiator caspase *dronc* and the effector caspases *drice* and *dcp-1*, and culminating with induction of the IAP-antagonists *reaper* and *hid*. This sequence of gene expression is differentially-regulated by proteins within the ecdysone hierarchy. Altogether, our results demonstrate that caspase activation during programmed cell death of the *Drosophila* larval salivary glands requires a coordinate and sequential transcriptional upregulation of both caspases and IAP-antagonists, providing important new insights into our understanding of the genetic control of caspase activation during an endogenously-regulated death response.

254B Using DGRP sequenced genomes to identify modifiers of cell death in *Drosophila* eyes *Jacob Khoussine*, Gary Cox, J. Ross Ogden, Daniel Tinney, James Thompson. Department of Biology, Univ. of Oklahoma, Norman, OK.

Quantifying phenotypic variation in a trait is a sensitive way to assess the role a genetic or environmental factor has on a targeted developmental process. Using the sequenced lines developed by Trudy Mackay and colleagues for the *Drosophila* Genetic Reference Panel (DGRP), we have combined genomic technology and electron microscopy to allow a precise assay measuring modifier effects on cell death in eye-facet development. The approach focuses on heterozygous effects, since half of the genome is from the *Basc* stock carrying the dominant mutation *Bar* and the other half constitutes autosomes of each sequenced DGRP strain. Heads were removed, bisected between the compound eyes, and mounted on SEM plugs. Plugs were sputter-coated with gold-palladium and scanned to provide high-resolution images to count facet number in each eye. Average facet number was calculated for each semi-genome from each DGRP line, and pairs of eyes from the same individual were also tracked. Comparing both eyes from the same individual allowed the additional measurement of developmental homeostasis as reflected in fluctuating asymmetry, $FA = |L - R|/((L + R)/2)$. DGRP lines differ significantly in alleles at loci affecting the degree of cell death. For example, for strain 25175, the average facet count was 105.4 ± 17.4, compared to strain 25174, the average was 141.0 ± 7.7 (significantly different by t-test). Thus, different DGRP genotypes differ in their effect upon cell death in eye facet number. These results will be discussed in the contexts of modifier loci for cell death and the effect of this disruptive process on developmental homeostasis.

255C Effect of Adenosine Signaling on Apoptosis in Imaginal Disc Cells *Lucie Kucerova*, Roman Sidorov, Michal Zurovec. Biology Centre CAS, Institute of Entomology, Ceske Budejovice, Czech Republic.

Adenosine (Ado) is a crucial metabolite and signaling molecule, which affects energy homeostasis and is released from cells under stress conditions. We showed earlier that an excess of extracellular Ado caused apoptosis of *Drosophila* imaginal disc cells *in vitro*. Our earlier results also suggested a key role of *Drosophila* adenosine receptor (DmAdoR) for the survival of certain types of somatic mosaic clones. In this study we analyzed the response of imaginal disc cells *in vitro* to extracellular adenosine using microarrays. We also induced different types of somatic mosaic clones in imaginal wing discs by flippase-FRT recombination system and MARCM labeling, and followed the survival of various clones in time. Our results revealed that the adenosine exposure of imaginal disc cells *in vitro* lead to the massive uptake of extracellular adenosine to the cells, which caused the downregulation of pantothenate and CoA biosynthesis and loss of mitochondrial membrane potential. The CoA is key precursor required for many biosynthetic reactions involved in lipogenesis. Interestingly clonal analysis *in vivo* showed that DmAdoR is required for survival of some types of mosaic clones, including the clones forming benign tumors *wts-/-* or *dco-/-*. Our results revealed that most of such clones are eliminated by apoptosis within 48 hrs after flippase induction. Interestingly, the clone survival can be rescued by the overexpression of adenosine deaminase. Taken together our results show that adenosine signaling is an important regulator of imaginal disc survival and energy homeostasis.

256A *spargel/dPGC-1* knockdown protects epithelial cell death in the wings like a proapoptotic gene. *Tomilowo Abijo*, Atanu Duttaroy. Biology, Howard University, Washington DC, DC.

Drosophila *spargel* a homologue of mammalian Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1

(PGC-1), is the terminal effector of the insulin signaling pathway. Ubiquitous *spargel* ablation causes reduced body size and sterility while overexpression of spargel in the midgut increases life span. From an unrelated genetic screen we first noticed that *spargel* knocked down in the wings causes large brown blemishes to appear on the wings. Appearance of those blemishes increase in size with age and the wings remain in held out position. Interestingly, similar type of blemishes appears on the wing in mutant clones of proapoptotic genes including *dronc*, *dark*, *grim, hid* and *reaper*. So, we hypothesize that loss of spargel function may have a protective effect on wing cell death. Further investigations using DAPI staining, TUNEL assay, live-cell imaging and ultrastructural analysis supports this hypothesis as fewer cell deaths occurred in the wings following the ablation of spargel. Since similar phenotype was obtained with multiple RNAi constructs of spargel therefore any possible off target effect was ruled out. These studies help us to confirm that *spargel/dPGC-1* is a propapoptotic gene responsible for epithelial cell death in the wing. The mammalian PGC-1 is a 3-isoform family of transcriptional coactivator that has been shown to play critical roles in oxidative metabolism via mitochondrial biogenesis. Moleculargenetic analysis of PGC-1 in mice and other vertebrate models were proven difficult because of the redundancy effects. Involvement of spargel in some novel biological functions in Drosophila is creating the opportunity to test them in vertebrates.

257B ABC Transporters are required for nurse cell corpse clearance in *Drosophila melanogaster.* *Clarissa S Santoso*, Kim McCall. Cell and Molecular Biology, Boston University, Boston, MA.

The final step of programmed cell death involves recognition and engulfment of the dying cells by phagocytic cells. Genetic studies in *Drosophila melanogaster*, *Caenorhabditis elegans*, and mammals have identified two evolutionarily conserved signal transduction pathways that act redundantly to regulate engulfment: the CED-1/-6/-7 and CED-2/-5/-12 pathway. Of these cell death abnormal (CED) proteins, the ABC transporter CED-7 is the only protein reported to be required in both the engulfing cell and the dying cell. However, its function in the cell death process remains the most enigmatic and the *ced-7* ortholog has not been identified in *D. melanogaster*. Homology searches revealed a family of putative *ced-7* orthologs that encode ABC transporters in *D. melanogaster*. To determine which of these genes functions similarly to *ced-7*, we analyzed their engulfment function in oogenesis, during which 15 germ cells in each egg chamber undergo programmed cell death and are removed by neighboring phagocytic follicle cells. It has been shown that genetically knocking down individual engulfment genes results in inefficient clearance of the germ cells, which then persist in late stage egg chambers. Only two of the putative *ced-7* genes are expressed significantly in the ovary, and we have characterized these using transposon insertions, deficiencies, and RNAi knock-downs. Genetic analysis strongly suggests that one of them, which we call *dmced-7* is necessary for germ cell clearance in the *D. melanogaster* ovary. Immunostaining shows that genetically knocking down *dmced-7* results in uncleared germ cells which persist in late stage egg chambers. Altogether, our findings suggest that CED-7 plays an evolutionarily conserved role in engulfment in *D. melanogaster*.

258C Identification of novel regulators of apoptosis *Alicia Shields*, Michelle Conti, Andreas Bergmann. Molecular, Cell & Cancer Biology, University of Massachusetts Medical School, Worcester, MA.

The goal of this project is to identify novel regulators of apoptotic cell death. Although several genes that play central roles in apoptosis have been known for many years, it is not clear that all the important players of cell death have been discovered. The initiator caspase, Dronc, the APAF-1 related protein that partners with Dronc to form the apoptosome, dArk, and the effector caspase, drICE are all recessive modifiers of apoptosis. Therefore, to capture recessive suppressors of apoptosis, we expressed the pro-apoptotic factor, *hid*, in the *Drosophila* eye using the *GMR*-promoter (*GMR-hid*) to generate a strong eye-ablation phenotype and induced genetic mosaics using the *ey-FLP/FRT* system. Using this method, we identified several recessive suppressors of apoptosis. This study aims to characterize 6 mutations that exhibit moderate to strong suppression of *GMR-hid* eye-ablation. Four of the six mutations are located on chromosome arm 2R, one is located on chromosome arm 3L, and one on chromosome arm 3R. Preliminary characterization of these mutations suggest that they play a role in developmental apoptosis and may act either in parallel or downstream of caspases in the apoptotic pathway. Complementation analyses to identify the genes in which these mutations occur are ongoing.

259A Cell Cycle Regulation of Apoptosis in *Drosophila melanogaster* *Ananya Chakravarti*[1], Bingqing Zhang[2], Shahina Maqbool[3], Sonam Mehrotra[4], Brian Calvi[1]. 1) Biological sciences, Bloomington, IN; 2) Advanced Cell Diagnostics, California 94560; 3) Albert Einstein College of Medicine, Bronx, New York 10461; 4) Center of Excellence for Epigenetics, Pune, India 411008.

The primary goal of this research is to understand how the cell cycle and apoptotic pathway intersect in multicellular animals using *Drosophila melanogaster* as a model organism. Our prior studies showed that cells in a specialized cell cycle, called the endocycle (G/S cycle), do not apoptose in response to genotoxic stress. This repression of apoptosis was common to all tissues composed of endocycling cells in the larva and adult, e.g. larval salivary glands and adult ovary. We have previously shown that the repression of apoptosis in endocycling cells of the larval salivary gland is

due to epigenetic silencing of the pro-apoptotic genes at the H99 locus and degradation of the p53 protein. To delve deeper into the mechanism that links cell cycle and apoptosis, we previously used microarrays to compare the gene expression of endocycling cells to mitotic cells. Among the differentially regulated genes, the target genes of the Myb-MuvB and E2F1 transcription factor complexes were expressed at significantly lower levels in the endocycling cells. Given the important role of the Myb-MuvB complex in the progression of cell cycle, we are currently evaluating its role in the regulation of apoptosis as well. We are also exploring the role of various chromatin modifiers for chromatin silencing of pro-apoptotic H99 genes during the endocycle. Since, apoptosis is an important barrier to oncogenesis, our findings are broadly relevant to understanding tissue specific response to cancer treatments.

260B Overgrowth promoting role of *Drosophila* macrophages *Neha Diwanji*[1], Caitlin Fogarty[1], Jillian Lindblad[1], Meghana Tare[1], Alla Amcheslavsky[1], Yun Fan[2], Andreas Bergmann[1]. 1) University of Massachusetts Medical School, Worcester, MA, USA; 2) University of Birmingham, UK.

Apoptosis-induced compensatory proliferation **(AiP)** is a mechanism that is involved in maintaining tissue homeostasis after massive stress-induced cell death. In this phenomenon, the dying cells induce proliferation of the surviving cells to compensate for the loss, and thus restore organ size. Along with wound healing and tissue regeneration, AiP also contributes to tumor repopulation following radiation or chemotherapy, where the dying tumor cells promote proliferation of the surviving tumor cells. Using a hyperplastic overgrowth tumor model (**"undead tissue"**) in *Drosophila melanogaster*, studies have identified several factors involved in AiP. Here we show that *Drosophila* epithelial undead tissue secretes **extracellular Reactive Oxygen Species (eROS)**, which attract and activate **hemocytes**, ***Drosophila* macrophages**. We have observed that these tumor-associated macrophages are necessary for inducing the overgrowth of undead tissue. Additionally, hemocytes secrete *Drosophila* TNF homolog **Eiger** that signals through the TNF receptor **Grindelwald** to active Jun-N-terminal kinase (**JNK**) in the undead tissue, and induce proliferation. We propose that there is a complex interplay between the tumor and its microenvironment that causes recruitment of macrophages, and **these tumor-associated macrophages signal to trigger proliferation of the epithelium**, thus promoting inflammation-induced tumorigenesis.

261C Role of hemocyte autophagy in modulating inflammatory responses to infection in the fat body *Mobina Roshandell*, Nuzhat Islam, Manisha Prasad, Catherine Brennan. Dept of Biological Science, California State University, Fullerton, Fullerton, CA.

The hemocytes and fat body constitute the primary immune sensing and response axis in *Drosophila*. Bidirectional signaling between these tissues determines the type, magnitude, and duration of response to different immune challenges. Curiously, we have found that genetic manipulation of the nutrient status and autophagic program of the hemocytes has a huge effect on the type and magnitude of the fat body inflammatory response to bacterial infection. Autophagy effectors are known to modulate the mammalian immune response in multiple ways including both regulating the degradative capacity of the phagosome and controlling the post-transcriptional regulation of cytokine release. Our findings that abrogating the autophagic capacity of hemocytes leads to massive over-induction of inflammatory responses in the fat body, but only during infection, suggest that, in *Drosophila*, autophagy in hemocytes suppresses the production of cytokine-like signals. Immunofluorescence analysis further supports our model that this effect is not mediated by effects on phagosome function. We will present the result of our efforts to identify the autophagy-dependent signal produced by hemocytes during bacterial infection.

262A Hemocytes promote a local Antimicrobial Peptide response in the Respiratory Epithelium and Fat Body of adult *Drosophila* *Rowan Baginsky*[2], Leire Herboso[2], Katrina Gold[2], Kalpana Makhijani[2], Brandy Alexander[2], Katie Woodcock[4], Elodie JV Raymond[3], Bruno Lemaitre[3], Frederic Geismann[4,5], Katja Brückner[1,2]. 1) Broad Center of Regeneration Medicine and Stem Cell Research; 2) Department of Cell and Tissue Biology, University of California, San Francisco; 3) EPFL Lausanne, Switzerland; 4) Kings College, London; 5) Memorial Sloan Kettering Cancer Center, New York.

Hemocytes play a crucial role as the cellular arm of the innate immune system in adult *Drosophila melanogaster*, specifically through their phagocytic capabilities. Their roles in other aspects of immunity, such as the induction of local antimicrobial peptide (AMP) responses, remain less defined and are the focus of our study.

Examining the anatomical locations of hemocytes by cryosectioning, we found that the majority of hemocytes in adult *Drosophila* accumulate along the respiratory epithelia (tracheal air sacs) of the thorax and head. This pattern emerges with developmental timing, as hemocytes relocate to these areas after completing phagocytosis of residual larval fat body cells in the young adult. The pattern also intensifies following infection with Gram-negative or Gram-positive bacteria, consistent with the accumulation of injected particles along the tracheal air sacs of the thorax and head. Here we propose that, in addition to functioning as phagocytosing macrophages, adult hemocytes relay the humoral immune response in anatomical locations adjacent to their sites lining the tracheal air sacs. Specifically, we found that induction of the AMP Drosocin relies on the presence of hemocytes in the adult; it was identified by examining infection-induced expression of several AMPs in genetically hemocyte-ablated flies. Interestingly, and

matching these major sites of hemocyte localization, a transgenic Drosocin-GFP reporter shows region-specific expression in the tracheal air sacs and the overlying fat body layers of the head and thorax, independent of the site of septic injury. Using tissue-specific in vivo RNAi, we confirmed that the respiratory epithelium and fat body are major contributors of Drosocin in response to gram-negative infection. Tissue specific RNAi analyses further indicate a requirement for IMD pathway signaling in both hemocytes and tracheal tissues for the induction of Drosocin following infection. Taken together, we propose hemocytes as sentinels of infection, which relay signals locally to adjacent immune tissues such as the respiratory barrier epithelium (tracheal air sacs) and the overlying fat body tissue of the head and thorax.

263B The Drosophila Chitinase-Like Protein IDGF3 is involved in Protection against Nematodes and in Wound Healing *U. Theopold*[1], L. Kucerova[1,2], B. Arefin[1], H. Maaroufi[1,2], H. Strnad[3], M. Zurovec[2]. 1) Molecular Biosciences, Stockholm University, Stockholm, Stockholm, Sweden; 2) ASCR, Institute of Entomology, University of South Bohemia, Ceske Budejovice, Czech Republic; 3) Institute of Molecular Genetics of the ASCR, Prague , Czech Republic.

Although human chitinase-like proteins (CLPs) are upregulated during several human disorders that affect regenerative and inflammatory processes, very little is known about their normal physiological function. Recently CLPs have been implicated in the control of nematode infections. We use infection with insect-pathogenic nematodes and wounding assays to study the immune and regenerative function of an insect CLP (Imaginal disc growth factor3, IDGF3). Like their vertebrate counterparts insect nematodes force their entry into the host via epithelial surfaces thus creating wounds. Transcriptome analysis was used to identify genes that are regulated in an IDGF3-dependent manner. Our results show that IDGF3 serves as an essential component required for the formation of hemolymph clots that seal wounds made by the nematodes and Idgf3 mutants display an extended developmental delay during wound healing. During nematode infections IDGF3 induces selected immune effectors and represses wingless and JAK/STAT signaling. Altogether our findings indicate that vertebrate and invertebrate CLP proteins function in analogous settings having broader impact on inflammatory reactions and infections. This opens the way to further genetic analysis of *Drosophila* IDGF3 and will help to elucidate the exact molecular context of CLP function.

264C *miR-34* Modulates Innate Immunity and Ecdysone Signaling in *Drosophila* *Xiao-Peng Xiong*[1], Krishna Kurthkoti[1], Kung-Yen Chang[2], Xingjie Ren[2], Jian-Quan Ni[3], Tariq M. Rana[3], Rui Zhou[1]. 1) Tumor Initiation and Maintenance Program; Development, Aging and Regeneration Program, Sanford-Burnham Prebys Medical Discovery Institute, La Jolla, CA; 2) Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA; 3) Gene Regulatory Laboratory, School of Medicine, Tsinghua University, Beijing, China.

MicroRNAs are endogenous small regulatory RNAs that modulate myriad biological processes by repressing target gene expression in a sequence-specific manner. Here we show that the conserved miRNA *miR-34* regulates innate immunity and ecdysone signaling in *Drosophila*. *miR-34* over-expression activates antibacterial innate immunity signaling both in cultured cells and *in vivo*, and flies over-expressing *miR-34* display improved survival and pathogen clearance upon gram-negative bacterial infection; whereas *miR-34* knockout animals are defective in antibacterial defense. In particular, *miR-34* achieves its immune-stimulatory function, at least in part, by repressing the two novel target genes *Dlg1* and *Eip75B*. In addition, our study reveals a mutual repression between *miR-34* expression and ecdysone signaling, and identifies *miR-34* as a node in the intricate interplay between ecdysone signaling and innate immunity. Lastly, we identify *cis*-regulatory genomic elements and *trans*-acting transcription factors required for optimal ecdysone-mediated repression of *miR-34*. Taken together, our study enriches the repertoire of immune-modulating miRNAs in animals, and provides new insights into the interplay between steroid hormone signaling and innate immunity.

265A Using the *Drosophila* Genetic Reference Panel to identify novel genes utilized in the immune response to the West Nile virus subtype Kunjin virus *Laura Ahlers*, Grace Carrell, Alan Goodman. School of Molecular Biosciences, Washington State University, Pullman, WA.

Insect-borne viruses, such as the flavivirus West Nile virus (WNV), pose a serious threat to public health worldwide. WNV became endemic to the United States in 1999, and now causes over 2,000 domestically acquired cases in the U.S. annually. At this time, there are no commercially available vaccines or therapeutics to treat WNV infections. Because of the reliance on a mosquito vector for the transmission of WNV to humans, it is imperative that we understand and control the insect vector to prevent mosquito-to-human virus transmission. In this work, we utilize *Drosophila melanogaster*, a well-established model for innate immunity, to elucidate the antiviral immune response in an insect. We use Kunjin virus (KUNV), a naturally attenuated subtype of WNV in our studies. We screened for novel components of insect immunity utilizing the *Drosophila* Genetic Reference Panel (DGRP). The DGRP is a panel of wild-type inbred fly lines whose genomes have been sequenced. This living library incorporates sufficient genetic diversity to reveal genetic variants that contribute to increased susceptibility or resistance to KUNV infection. We determined the mortality rate for each fly line and used it for subsequent Genome-Wide Association

Study (GWAS). Through this research we identified the genes *Mustard*, *Pecanex*, and *Thisbe* for ensuing studies. We selected these genes based on their high significance values and putative contribution to immunity. We will validate the contribution of each of these candidate genes to the immune response to KUNV. Future work will determine if these genes are also necessary for the mosquito response to KUNV.

266B Production and detection of Vago and virus induced RNA-1 (vir-1) in *Drosophila melanogaster* using monospecific antisera during Nora virus infection *Wilfredo Lopez*, Brad Ericson, Darby Carlson, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Monospecific antisera production is used to detect and characterize proteins of interest for functional annotation. In *D. melanogaster*, the genes *Vago and virus induced RNA-1* (*vir-1*) are implicated in innate immunity during Nora virus infection. However, the antiviral mechanism that Vago is involved in is not fully understood and the role of vir-1 within this mechanism has not been determined. Vago and vir-1 were produced from synthetic genes that were codon optimized for expression in *E. coli*. CD-1 Swiss outbred female mice were injected with either the Vago or vir-1 protein for monospecific antisera production. Western Blot analysis showed that the antisera reacted specifically with Vago and vir-1. These same antisera were used to successfully detect Vago and vir-1 during Nora virus and Drosophila C virus (DCV) infection in approximately thirty day old flies. DCV was used as a control for Nora virus to determine if DCV could stimulate Vago and vir-1 production. To evaluate detection of Vago, flies were aged over twenty-seven days and examined at three day intervals. Vago antiserum was able to successfully detect Vago at all three day intervals in the twenty-seven-day time span. In the time course experiment, monomeric Vago protein was detected in the early portion of the trial, but subsequently was found only in oligomerized forms. These results indicate that Vago may oligomerize throughout the course of Nora virus infection to become active and function in an unknown antiviral mechanism. Ultimately, producing monospecific antisera for Vago and vir–1 proteins will allow for continued work in determining these proteins antiviral mechanisms. 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.

267C Pathogenicity of Nora virus in Germ-free *Drosophila melanogaster* *Makayla Nemecek*, Rebecca Best, Shelby Peters, Carlie Prososki, Lesley Towery, Brad Ericson, Darby Carlson, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Microbiota in mammalian species plays a key role in the presence of gastrointestinal viruses. This is also true for invertebrate species, even though they are not as widely characterized. One such invertebrate virus that replicates in the gut and may have a connection with healthy microbiota is *Drosophila melanogaster* Nora virus. Nora virus is a picornavirus that exhibits fecal-oral transmission, and demonstrates persistent infection without any characterized pathogenicity. These characteristics, along with data that demonstrates the microbiota of the gut of both vertebrate and invertebrate species may be dependent upon persistent viral infection, led us to hypothesize that Nora virus may be important in maintaining a healthy gut microbiota in *D. melanogaster* allowing them to live longer. Germ free *Drosophila* were generated with the use of antibiotics and divided into four separate treatment groups: Nora virus positive/bacteria positive, Nora virus negative/bacteria positive, Nora virus negative/bacteria negative, and Nora virus negative/bacteria negative. The presence of Nora virus was determined via RT-PCR, using gene specific primers. The presence of microbiota was determined by homogenizing *D. melanogaster* in Luria broth (LB) and plating on LB agar plates. After all treatment groups were tested, a longevity study was conducted on each of the conditions. The longevity study on the *Drosophila* that possessed their normal microbiota suggests that Nora virus does not enhance longevity, but microbiota is needed when virus is present to live. However, data suggested that when no virus is present, microbiota can actually hinder the longevity of *D. melanogaster*. This suggests that Nora virus may not be needed to maintain a healthy gut microbiota and microbiota may only be beneficial when Nora virus is present, but further testing is needed. 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.

268A Precise Regulation of Developmental Apoptosis in *Drosophila* Neural Stem Cells *Antoine Borensztejn*[1,2], Kate Harding[2], Richa Arya[1,2], Kristin White[1,2]. 1) Massachusetts General Hospital, Charlestown, MA; 2) Harvard medical School, Charlestown, MA.

The control of cell number is essential for the proper formation of organs during development. In the developing *Drosophila* central nervous system, subsets of neural stem cells die at the end of embryogenesis. In each of the central abdominal hemisegments of the ventral nerve cord 27 out of 30 neural stem cells (neuroblasts) undergo programmed cell death. Thus only 3 neuroblasts per abdominal hemisegment survive until larval life. When this apoptosis is inhibited, these stem cells continue to divide and give rise to a hyperplastic central nervous system and adult lethality. Our lab previously showed that Notch and AbdominalA control the transcriptional activation of the central cell death regulators *grim* and *reaper* in the doomed neuroblasts, ensuring their elimination through the

canonical apoptotic pathway. Our current studies focus on the factors that confer resistance to apoptotic signals in the 3 surviving neuroblasts, and on further identification and characterization of the pro-apoptotic signaling occurring in the dying neuroblasts. Many additional regulators of neuroblast apoptosis have been identified in an RNAi screen. These studies will contribute to the overall understanding of the mechanisms controlling the activation of developmentally important apoptosis in the nervous system.

269B Identifying Enhancers in a Novel Cluster of Immune-Responsive Genes in *Drosophila melanogaster* *Marley Hilleger*, Zeba Wunderlich. University of California, Irvine, Irvine, CA.

Insects, e.g. *Drosophila melanogaster*, lack an adaptive immune system and are dependent on their innate immune response to fight pathogens. Following infection, Toll and IMD signaling pathways are triggered and activate the expression of antimicrobial peptides (AMPs) and other effector proteins. AMPs are crucial for pathogen clearance; however, the deletion of a single AMP gene often has no effect on survival, due to functional redundancy between genes. This redundancy makes it hard to study the differences in AMP expression between individuals that may affect animal immune response. Clemmons et al., have recently characterized a family of twelve genes called *Bomanins* (*Boms*), which encode AMP-like peptides. The deletion of a region containing ten *Boms* completely ablates the Toll-mediated immune response, making this region uniquely suited to studying the effects of AMP expression on survival following infection. We hypothesize that this *Boms* cluster contains novel immune-responsive enhancers responsible for driving Toll-dependent transcription. To identify enhancers within the *Boms* cluster, we computationally identified intergenic regions with an abundance of binding sites for immune-responsive transcription factors (three Rel/NF-kB-family factors and one GATA factor). We cloned these regions into luciferase reporter constructs and transfected them into *D. melanogaster* S2* cells, a macrophage-like cell line. We stimulated an immune response in these cells using heat-killed bacterial cultures then measured luciferase expression. Preliminary results suggest that several of the intergenic regions within the *Boms* cluster contain enhancers. The *Boms* cluster represents an understudied yet essential aspect of the Toll-mediated response. Identifying the regulatory elements within this cluster is critical to understanding how changes in regulatory DNA affect an animal's immune response.

270C Role of brain-specific NF-κB-dependent mmunity in Drosophila lifespan determination and age-related neurodegeneration *Stanislava Chtarbanova*[1,3], Ilias Kounatidis[2], Yang Cao[3], Margaret Hayne[3], Dhruv Jayanth[2], Barry Ganetzky[3], Petros Ligoxygakis[2]. 1) Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Department of Biochemistry, University of Oxford, Oxford, UK; 3) Department of Genetics, University of Wisconsin, Madison, WI.

Age is a major risk factor for many diseases, including neurodegenerative diseases, which are among the leading causes of long-term disability and death in the United States and developed countries and are currently untreatable. In aging individuals, elevated levels of pro-inflammatory cytokines result in a chronic inflammatory status, known as inflammaging. This remains subclinical in many elderly individuals; however, some others go on to develop age-dependent neurological conditions. Thus, identifying the factors that predispose those developing disease or what distinguishes those that "age well" remains a challenge, as restoring or preserving physiological functions of an organism is of substantial clinical importance. We used Drosophila to explore the potential cost of immunity in the ageing brain. We showed that NF-κB-dependent transcription of antimicrobial peptides (AMPs) increased with age and that this was accompanied by progressive locomotor decline and neurodegeneration. Genetic analysis revealed that mutations in three negative regulators of the Drosophila IMD signaling pathway namely, *pirk*, *trabid* and *transglutaminase* resulted in constitutive immune activation with high levels of AMPs in heads and brains. These mutants exhibited shorter lifespan accompanied by severe neurodegeneration and locomotor defects, when compared to controls. In *trabid* mutants, all these phenotypes were suppressed when the NF-κB transcription factor Relish was silenced in glia. Moreover, reducing normal levels of IMD signaling in the brain of healthy, wild-type flies resulted in lifespan extension with increased locomotor activity in old age accompanied by increased hormonal signaling and ameliorated metabolic profile. Altogether our results demonstrate that the IMD/NF-κB/Relish immune signaling in glia is important for both healthy ageing and age-dependent neurodegeneration.

271A Physical interactions between larval hemocytes and fat body are affected by infection and nutrient status, with regulation of the interaction depending on different pathways in the two tissues, as well as on metabolic and immune status of the animal *Eniola Ogundipe*, Kristofer Serrano, Catherine Brennan. Biological Science, California State University, Fullerton, Fullerton, CA.

The fat body is the primary metabolic organ of the fly larva, storing energy as lipids, and releasing it under the control of developmental, metabolic, and inflammatory cues. The fat body is also an immune organ, secreting antimicrobial peptides and other molecules into the hemolymph during infection. In addition to these direct effector functions, the fat body plays regulatory roles, co-ordinating both growth and systemic immune responses in the whole larva. The other main immune tissue is the system of circulating and sessile hemocytes. While research has revealed extensive bi-directional signalling between the two tissues in the context of metabolic and immune

challenge, we have focused our attention on the physical interactions between hemocytes and fat body. Using a combination of tissue-specific gene manipulation, fluorescent microscopy, and qPCR analysis of gene expression, we describe patterns of hemocyte recruitment to the fat body in response to infection and starvation, and the concomitant transcriptional responses in the fat body. We find that although starvation and immune challenge both lead to hemocyte aggregation at the fat body, the regulatory mechanisms are not the same, not surprisingly. Moreover, by manipulating gene expression in the hemocytes vs the fat body, we are dissecting the distinct contributions to these two tissues to hemocyte recruitment. Since obesity in mammals leads to the accumulation of pro-inflammatory macrophages in adipose tissue, which directly contributes to the pathologies associated with metabolic syndrome, using the fly to analyse phagocyte-adipose interactions will be useful towards deepening our understanding of this medically important phenomenon.

272B Dynamic interplay between bacterial growth and the host immune response generates a stochastic outcome of infection David Duneau[1,2], Jean-Baptiste Ferdy[2], Jonathan Revah[1], Hannah Kondolf[1], Gerardo Ortiz[1], Brian P. Lazzaro[1], *Nicolas Buchon*[1]. 1) Cornell Institute of Host-Microbe Interactions and Disease, Cornell University, Ithaca, NY; 2) UMR5174, Laboratoire Evolution et Diversite Biologique.

A central problem in infection biology is understanding why two individuals exposed to apparently identical infections may have dramatically different clinical outcomes. One individual may recover fairly easily while the other suffers devastating illness or even death. We have developed an experimental model where genetically identical, co-housed fruit flies given identical systemic infections experience dramatically different outcomes, with some individuals succumbing to lethal acute infection while others control the pathogen as a fairly asymptomatic persistent infection. We found that flies die at a fixed bacterial burden regardless of the time post-infection at which death occurs, and that the lethal burden varies across distinct bacteria. Using multiple experimental and genetic manipulations, we identify 3 key factors that predict whether the infection proceeds to this lethal burden. The first is the effective rate of bacterial proliferation, which is the difference between the rate of cell division and the rate of clearance by the immune system. The second is the establishment of effective immunological control. In particular, we have found that the activation of the Imd pathway can vary dramatically between individuals, suggesting that the Imd pathway is intrinsically noisy and that inter-individual stochasticity early in infection might lead to differences in the probability of ultimate survival. Third, we infer a threshold pathogen density that must be reached to enable the switch to a lethally acute infection instead of a chronically persistent one. Inter-host variation in survival must therefore originate in the ability of the pathogen to reach that threshold before effective immune control is established. Altogether, our results illustrate the mechanisms underlying the variable nature of infection outcome and provide a framework for studying the individual host-pathogen parameters that govern the progression of infection and lead ultimately to life or death.

273C Investigating the impact of chronic infection on male reproduction in *Drosophila melanogaster* *M. Chambers*[1], K. Nichols[2], K. Staub[2], S. Tener[2]. 1) Biology, Bucknell University, Lewisburg, PA; 2) Biology, Muhlenberg College, Allentown, PA.

Environmental changes that alter the fitness of an organism are important because they determine which traits continue to subsequent generations. Using *Drosophila melanogaster* as a model organism, we are investigating the relationship between male fitness and infection with the natural pathogen *Providencia rettgeri.* If a fly survives acute infection with this bacterium, the fly is chronically infected for the remainder of its life. Little was known, however, about how chronic infection impacts male reproduction. We find that chronically infected male flies produce fewer offspring when mated to groups of virgin females. We examined the mechanism behind this reproductive defect by testing the impact of chronic infection on the basic ability to court and copulate, sperm depletion and replenishment, and the ability to compete with other males.

We find that male flies chronically infected with *P. rettgeri* exhibit no reproductive defect when mated in an isolated pair with a virgin female – these pairings result in the same number of viable adult offspring, and there is no significant different in time until mating and duration of mating. We also find that sperm replenishment is not affected when chronically infected male flies are successively mated with virgin females. These results suggests that sensory detection of illness and subsequent mate choice is responsible for the fitness defect seen in chronically infected male flies.

Work across species suggests that organisms have evolved mechanisms to detect the immunological strength of a prospective mate. Mate choice is influenced by the composition of a male's cuticular hydrocarbons (CHCs), which are long hydrocarbon molecules found on the external surface of most insects that mediate a variety of host processes including desiccation resistance, communication, and mate choice. We have tested both mate choice and CHC signatures across multiple infections capable of establishing chronic infection. For competitive mating assays, the bacterial load of the infected male fly was determined to assess whether infection severity influences mating success. Using genetic mutants in select metabolic pathways, we will determine how infection influences these phenotypes. Ultimately, our research will provide a mechanistic example of how sensory detection of infection status influences mating success.

274A How EF, a cAMP-inducing toxin from *Bacillus anthracis*, blocks Rab11-dependent trafficking *Annabel Guichard*[1], Prashant Jain[1], Mahtab Moayeri[3], Curtis Sera[1], Jammal Abu-Kazneh[1], Ruth Schwartz[1], Victor Nizet[2], Stephen Leppla[3], Ethan Bier[1]. 1) Dept Biol, Univ California, San Diego, La Jolla, CA; 2) Dept. Pediatrics Univ. California, San Diego, La Jolla, CA; 3) Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD.

In the past decade, we have developed a *Drosophila* model for analyzing the activity of anthrax toxins. In this system, conditional expression of Edema Factor (EF) or Lethal Factor (LF) in specific tissues leads to defined phenotypes that provide clues for identifying target pathways. Thus, toxin-induced wing phenotypes led to the identification of Rab11/exocyst-dependent trafficking as a target of both EF and LF. As a result, cargo proteins such as Cadherins, which normally depend on Rab11 for proper trafficking, fail to reach inter-cellular junctions in response to EF, potentially contributing to edema and vascular effusion during late phases of anthrax infection. In the present study, we provide further mechanistic analysis to connect cAMP over-production to Rab11 inhibition. We find that EF blocks Rab11 after the GTP-loading step. Both primary cAMP effectors, PKA and Epac, contribute to this effect, but act at distinct steps of the delivery process. In contrast, in mammalian systems, Epac and its partner Rap1 appear to be the primary effectors mediating the effects of EF, both in cell culture and *in vivo*. The small GTPase Arf6, which initiates endocytic retrieval of adhesion components, also contributes to junctional homeostasis by counteracting Rab11-dependent delivery of cargo proteins at sites of cell-cell contact. Importantly, chemical inhibition of key regulators of junctional trafficking offers potent protection against EF toxicity in cell culture and *in vivo*, opening new potential therapeutic avenues for treating symptoms caused by cAMP-inducing toxins.

275B *Coxiella burnetii* infection in *Drosophila melanogaster*: key factors in pathogenesis *Zachary Howard*, Reginaldo Bastos, Aoi Hiroyasu, Alan Goodman. School of Molecular Biosciences, Washington State University, Pullman, WA.

Coxiella burnetii is the causative agent of Q fever, a zoonotic disease that threatens both human and animal health. Due to the paucity of experimental animal models and the categorization of *Coxiella burnetii* as a Select Agent that requires BSL3 facilities, there remains a gap in our current knowledge regarding how host factors interface with bacterial components and affect pathogenesis. Here we used *Drosophila melanogaster*, in conjunction with the attenuated BSL2 Nine Mile phase II (NMII) clone 4 strain of *Coxiella*, as a model to investigate host and bacterial components implicated in infection. We demonstrated that NMII *Coxiella* is able to replicate and induce mortality in wild-type adult *Drosophila* despite an IMD-mediated immune response. While loss of IMD pathway components exacerbates infection, we observed no increase in bacterial load. We also show that in the absence of the *Drosophila* tumor necrosis factor (TNF) homolog, Eiger, *Coxiella*-infected flies exhibit reduced mortality to infection. Finally, the *Coxiella* type 4 secretion system (T4SS) is critical for the formation of the *Coxiella*-containing vacuole and establishment of infection in *Drosophila*. Altogether, our data revealed that the *Drosophila* TNF homolog Eiger and the *Coxiella* T4SS are implicated in the pathogenesis of the attenuated NMII strain in the animals. Additionally, the *Drosophila*/NMII clone 4 model mimics relevant aspects of the infection in mammals, such as a critical role of host TNF and bacterial T4SS in pathogenesis. Our work also demonstrates the usefulness of this BSL2 model to investigate both host and *Coxiella* components implicated in infection.

276C Comparative Transcriptomics of the *D. melanogaster* Response to Bacterial Infection Joo Hyun Im, Katia Troha, Jonathan Revah, Nicolas Buchon, *Brian Lazzaro*. Cornell Institute for Host-Microbe Interactions and Disease, Cornell Univ, Ithaca, NY.

Our understanding of the *D. melanogaster* transcriptional response to bacterial infection is based primarily on studies that have relied on a small number of microbes. We thus have little sense of how generic or specific the responses are. In the present study, we infected *D. melanogaster* with 10 bacteria that span a range from low to high virulence (*Micrococcus luteus*, *Escherichia coli*, *Serratia marcescens* Type, *Pectinobacterium* (*Erwinia*) *carotovora* Ecc15, *Providencia rettgeri*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Providencia sneebia*, *Serratia marcescens* Db11, and *Pseudomonas entomophila*). These bacteria include both Gram-positives and Gram-negatives, and all are frequently employed in studies of *Drosophila* immunity. We generated RNA-seq data at 12, 36, and 132 hours post-infection and tested for shared and unique transcriptional responses. We used principal component analysis (PCA) to cluster the responses to each infection. The first principal component explained 34% of the variance and was primarily driven by the magnitude of the Imd-pathway response. The second principal component explained 27% of the variance and was primarily determined by Gram-negative versus Gram-positive infection. We identified a robust core response of 166 genes that were differentially expressed in response to 7 or more of the 10 infections. Core induced genes carried Gene Ontology (GO) annotations including "immune response," "response to oxidative stress," "protein translation, modification, and secretion," "cellular homeostasis," and "iron ion transport/homeostasis," while repressed core gene annotations included multiple metabolic processes. Nevertheless, each infection stimulated an overall unique transcriptional signature. For example, 30% of the genes differentially regulated by *S. aureus* infection and 22% of

the genes regulated by *P. entomophila* infection were differentially regulated in response to those infections alone. In contrast, the highly virulent *P. sneebia* infection elicited a very small transcriptional response reminiscent of infection with the benign bacteria, consistent with previous reports that *P. sneebia* evades or suppresses the host immune response. Taken as a whole, our data allow the identification of an expanded core set of host responses to generic bacterial infection, as well as specific responses that give insight into the particular interactions between each pathogen and the host, and reveal hundreds of new genes that will serve as fodder for future investigation.

277A The transcription factor *CrebA* promotes disease tolerance upon bacterial infection *Katia Troha*, Joo Hyun Im, Jonathan Revah, Brian Lazzaro, Nicolas Buchon. Cornell Institute for Host-Microbe Interactions and Disease, Cornell University, Ithaca, NY.

In order to survive infection, a host needs both to control bacterial growth (resistance) and to mitigate the damage inflicted by infection (tolerance/resilience). To identify new, key biological processes required to survive infection, we used RNA-seq to profile the *D. melanogaster* transcriptomic response to 10 bacterial pathogens that span the spectrum of virulence. We found a set of 86 genes that were differentially expressed in all infections and thus constitutes a core response. Among these, we identified *CrebA* as a novel transcription factor involved in the host response to infection. *CrebA* is transcriptionally upregulated by all 10 bacteria, but it is not upregulated by sterile wound. We further demonstrated that *CrebA* expression is regulated in the fat body by both the Toll and Imd pathways upon infection. RNAi knockdown of *CrebA* expression in the whole fly or in the fat body alone significantly increased mortality from bacterial infection. However, this decrease in survival is not accompanied by an increase in bacterial load. Furthermore, we showed that *CrebA* RNAi flies succumb to infection at a lower pathogen burden than wild type flies. These data suggest that *CrebA* promotes disease tolerance. Altogether, we have identified a novel regulator of disease tolerance that is directly regulated by the Toll and Imd pathways and is required to survive bacterial infections.

278B Trade-offs between fecundity and survival in starvation-selected fly populations *Tammara Beeghly*, Allen Gibbs, Timothy Saitta, Laurel Raftery. School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV.

Evolutionary outcomes are determined by traits that affect an organism's reproduction and survival. These life history traits are reflected in an organism's physiology, development and behavior. The purpose of our research is to investigate population variation resulting from stress responses in flies. We used replicated starvation-selected populations as a model to identify differences in fecundity and survival compared to fed control flies. The starvation-selected flies have been maintained in the laboratory as reproductively isolated populations for more than 100 generations. Evolved differences between starvation-selected flies and fed controls include increased lipid storage. Despite having greater energy reserves, starvation-selected flies have lower fecundity than controls. The reduced fecundity could arise from an altered physiology, which limits the rate of mature egg production, or a change in development, resulting in reduced ovarian capacity for egg production. The goal of our initial experiments was to see if there is a trade-off between female fecundity and starvation survival. We found that females who laid fewer eggs survived starvation stress longer. Starvation-selected females have significantly fewer ovarioles per ovary. Because ovariole number is determined during the larval stages, our results show that selection on adult stress resistance has affected pre-adult developmental patterns. Future research will determine when and where these differences arise. Our research is supported by the NSF.

279C Physiological Evolution in Starvation-Selected Drosophila *Allen Gibbs*, Neha Parmar, Poonam Patel. School of Life Sciences, University of Nevada, Las Vegas, NV.

Experimental evolution uses replicated, outbred populations to study the process of evolution. Replicate populations generally evolve along similar trajectories, but can diverge over time due to drift or subtle differences in how populations are handled. We selected for starvation resistance in three populations of Drosophila melanogaster. After ~80 generations of selection, all populations survived >11 days without food, whereas unselected fed control flies survived less than four days. One starvation-selected replicate consistently survived over 24 hours longer than the other populations, and genomic data revealed significant divergence in SNP allele frequencies across the genome. To identify the physiological mechanisms underlying these differences, we analyzed the energy budgets of starvation-selected and fed control flies. Starvation survival can be increased by three non-exclusive physiological mechanisms. Starvation-selected flies can store more energy (e.g. lipids, carbohydrates) before selection is imposed, they can use energy less rapidly, or they can tolerate lower energy contents (i.e. utilize a greater proportion of initial energy stores before dying). Potential behavioral mechanisms include ingestion of microbes that have colonized corpses of flies who have already died during starvation selection ("cannibalism"), or acquiring energy from fecal material (coprophagy). We found that starvation-selected flies stored 3-4 times more energy than controls, acquiring it

during an extended larval feeding period. Their metabolic rates were ~25% lower, but they did not consume a greater fraction of initial lipid before death. Both starvation-selected and control flies survived longer in vials containing fly corpses, but selected flies were not “better” cannibals. The presence of large quantities of fecal material did not affect survival. In all assays, replicate populations did not differ significantly. We conclude that divergence in starvation survival is likely the result of relatively small changes in lipid storage and metabolic rate that were statistically undetectable, but together had a significant effect on starvation resistance. Supported by IOS-1355210 from NSF and R15-GM100395 from NIGMS.

280A Hypergravity, Endoplasmic Reticulum Stress, and the Unfolded Protein Response in *Drosophila*. *Ravikumar Hosamani*, Iman Hamid, Sharmila Bhattacharya. Space Biosciences Division, NASA Ames Research Center, Mountain View, CA.

Endoplasmic Reticulum (ER) stress is an imbalance between the load of proteins entering the secretory pathway and the protein-folding capacity of the ER. The Unfolded Protein Response (UPR) is a series of protein pathways which either re-establish ER homeostasis or, in cases of chronic or high-intensity ER stress, initiate apoptosis. In the present study, we aim to elucidate molecular and genetic mechanisms that respond to hypergravity-induced ER stress in *Drosophila melanogaster*. In a previous study from our lab, proteins related to Ca^{2+} regulation were significantly down-regulated in hypergravity (3g), leading to possible accumulation of intracellular Ca^{2+} which several reports suggest can lead to ER stress and autophagy. In a parallel study from our lab, hypergravity was also associated with significantly lower survival in adult male and female flies (2-3d old w^{1118} strain) compared to control (1g) flies. All flies were fed a tunicamycin-incorporated diet (12µM) which induces ER stress and, therefore, activates the UPR. These results indicate that hypergravity further exacerbates tunicamycin-induced ER stress in *Drosophila*. We hypothesize that the hypergravity-induced down-regulation of the aforementioned calcium-regulating proteins leads to elevated intracellular Ca^{2+} levels and, therefore, results in ER stress and mortality. In the current study, we focus on better understanding the genetic and molecular basis of hypergravity-induced ER stress. Preliminary survival data of RNAi mutants targeting upstream UPR proteins (Ire1, Xbp1, Perk, Atf4) suggest that Xbp1 in particular plays a sex-dependent role in the UPR response to hypergravity-induced ER stress. We also test hypergravity-induced changes in mRNA expression levels of UPR pathway-related proteins over time. Preliminary results show that, by 24 hrs, hypergravity can coordinate changes in expression levels of ER stress response genes for flies on tunicamycin.

281B The effects of altered lactate dehydrogenase expression on aging in *Drosophila melanogaster* *Dani Long*[1], Eileen Chow[1], Doris Kretzschmar[2], Jaga Giebultowicz[1]. 1) Integrative Biology, Oregon State University, Corvallis, OR; 2) Oregon Institute of Occupational Health Sciences, Oregon Health and Science University, Portland, OR.

Aging is accompanied by many changes in metabolic pathways of the brain including increased oxidative stress responses. We used RNA-seq to identify transcripts that had significantly different expression patterns in the heads of old flies compared to young flies. One of the genes identified was *ImpL3*, which encodes the *Drosophila* lactate dehydrogenase (LDH) enzyme. *ImpL3* is expressed at low levels in the heads of young *Drosophila* but its expression increases and becomes rhythmic in the heads of old flies maintained in 12:12 light/dark cycles. LDH reversibly interconverts pyruvate and NADH to lactate and NAD+. Lactate may be converted to pyruvate and enter the TCA cycle or it may be released into the extracellular space and utilized by other cells. Changes in LDH activity and lactate levels have been linked to tumor metabolism, long-term memory formation, and the metabolic coupling of glial cells and neurons in mammals. In flies, *ImpL3* expression is increased upon exposure to oxidative stress; however, it is not clear whether this increase is protective or detrimental. In order to understand the biological significance of increased *ImpL3* expression, we employed the TARGET system to over- or under-express *ImpL3* in the neurons, glia, perineurial glia or all clock expressing cells in the head via *tim*-Gal4. We determined that the over-expression of *ImpL3* within all clock cells significantly reduced lifespan. *ImpL3* over-expression in neurons or glia alone showed a modest shortening in median lifespan; however, overexpression in perineurial glia alone caused a significant reduction in lifespan, implicating these cells in premature mortality. The down-regulation of *ImpL3* by RNAi in neurons alone resulted in extended longevity of males but not females. The opposite effect of over-expression versus down-regulation of *ImpL3* in neurons on lifespan suggests that tight regulation of LDH activity is important in the aging brain. To investigate the effects of *ImpL3* on neuronal health we compared brain sections of old flies overexpressing *ImpL3* compared to age-matched controls. Our data suggest that the over-expression of *ImpL3* in neurons or glia alone leads to increase in vacuoles which are indicative of brain neurodegeneration. These data suggest that LDH activity in different cells of the brain may be tightly regulated. We are currently conducting biochemical studies to understand how age-related elevation of *ImpL3* expression affects LDH activity and metabolite levels and distribution in the aging brain.

282C Exposing Adult Flies to Daily Intoxicating Doses of Ethanol Affects their Midgut Morphology and the Tolerance of their Progeny to the Drug
Michael McPherson, Michelle Bonilla, *Mariano Loza-Coll*. Department of Biology, California State University, Northridge - Northridge, CA.

In recent years, there has been increased interest in the inheritance of enhanced metabolic responses to environmental conditions, such as diet, drug tolerance and addiction. While the available evidence points to epigenetic modifications of DNA (chromatin remodeling and DNA methylation) as two major underlying mechanisms of such inherited propensities, the source of epigenetic imprinting is less clear. Moreover, in mammals, it is difficult to separate the role of regulatory imprinting of gametes produced in parents subjected to a specific environmental condition from epigenetic modifications that occur directly in internally developing embryos. To circumvent this problem, we exploited the external development of *Drosophila melanogaster*. Female adults were exposed daily or every 48 hours to a partially intoxicating dose of ethanol for two weeks. After the exposure period, they were allowed to lay eggs for 3-5 days in the absence of any further intoxications, and their progeny was allowed to develop under standard (unexposed) conditions. The progeny from flies that were intoxicated daily remained active for several minutes longer than progeny from naïve parents when exposed to ethanol as adults, revealing an inherited higher tolerance to the drug. These observations suggest that prolonged exposure to ethanol in flies can somehow imprint tolerance to the drug in their progeny. In addition, daily ethanol exposure of the parents caused a significant alteration in the cell composition of their posterior midgut intestinal epithelium, consistent with alterations in the turnover of their intestinal stem cells.

283A The control of fat storage by splicing factor 2 (SF2) in *Drosophila*
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In Western societies where food is abundant, these excess nutrients are stored as fats mainly in adipose tissue. Fats are stored in structures known as lipid droplets, and a genome-wide RNAi screen performed in *Drosophila* cells has identified several genes that are important for the formation of these droplets. One group of genes found during this screen included those that regulate mRNA splicing. Previous work from our lab has identified a number of splicing factors that play a role in regulating fat storage; however, the full complement of splicing proteins that regulate lipid metabolism is still unknown. In this study, we describe a role for splicing factor 2 (SF2) in the control of *Drosophila* metabolism. Decreasing the levels of SF2 specifically in the adult fat body using RNAi resulted in higher triglyceride levels compared to the control flies. Consistent with this finding, SF2-RNAi flies survive longer when starved. To determine whether the fat accumulation phenotype observed in flies with less SF2 was due to increased feeding, food consumption was measured over a 24 hour period using the CAFÉ assay. Interestingly, the SF2-RNAi flies consumed less food than the controls, indicating that increased feeding was not the cause of the augmented fat storage in these flies. We next wanted to address the question of whether SF2 regulates the expression of genes important for lipid storage. To do this, quantitative PCR was used to measure the levels of CPT1, a major regulator of beta-oxidation. We chose CPT1 because it has been shown to be alternatively spliced into two major variants, one of which is more catalytically active than the other. In control flies, we observed higher levels of the more catalytically active CPT1 variant, while SF2-RNAi flies expressed roughly equal amounts of both variants. This suggests that there is less CPT1 activity in flies with decreased SF2 in their fat bodies potentially promoting the increased triglycerides in these animals. Together, this study identifies a novel splicing factor responsible for the regulation of lipid storage in the *Drosophila* fat body and contributes to our understanding of the mechanisms which influence the regulation of fat storage in adipose-like cells.

284B Evaluation of the effect of resveratrol on genotoxicity of nicotine, by wing spot test in *Drosophila melanogaster*
Mauro Magaña-Acosta[1], B. Gomez-Loza[1], M. Garfias[2], A. Browning[2], H. Perry[2], G. Federer[2], I.E. Dueñas-García[1], M.E. Heres-Pulido[1], N.A Velazquez-Ulloa[2]. 1) Genetic Toxicology, FES Iztacala, Universidad Nacional Autónoma de México, Los Barrios No.1, Los Reyes Iztacala, C.P. 54090, Tlalnepantla, Estado de México, México; 2) Biology Department, Lewis & Clark College, 0615 SW Palatine Hill Rd., Portland OR, USA, 97219.

Oxidative stress has been associated to lifespan shortening, aging and genotoxicity of chemical agents. Nicotine has the capacity to produce reactive oxygen species (ROS), which can promote damage on cellular and mitochondrial DNA, proteins and lipids. This has been recognized as one of the most important toxic mechanisms of nicotine. It has been suggested that the intake of antioxidants may help to prevent its effects. Resveratrol (RES) is known as a potent antioxidant. Several studies show that RES has antitumor and antiaging properties. We investigated, 1) the genotoxic effect of nicotine at 2 different concentrations [12.5 and 50 μM] dissolved in miliQ water, 2) the genotoxic effects of RES [11μM] dissolved in ethanol [2% v/v], 3) the effect in co-treatment of RES with the two different concentrations of nicotine [12.5 and 50 μM], and 4) if RES helps to decrease the genotoxic effects of nicotine. The treatments and co-treatments were tested with the *Drosophila* wing spot test in Standard (ST) and High Bioactivation (HB) crosses, with basal and high levels of cytochromes P450 (Cyp450s), respectively. In the ST cross RES [11 μM] was genotoxic, there was not genotoxicity for any concentration of nicotine, but nicotine [12.5 and 50

µM] co-treatments with RES [11 µM] were genotoxic. In the HB cross there was not genotoxic effect in any treatment. We conclude that at these concentrations the nicotine was not genotoxic; we propose that the genotoxicity differences between both crosses could be due to the different levels of Cyp450s, and we intend to clear their contribution in further studies.

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285C The regulation of lipid storage by TRA and TRA2 in *Drosophila* *Cezary Mikoluk*[1], Alexis Nagengast[2], Justin DiAngelo[1]. 1) Division of Science, Penn State Berks, Reading, PA; 2) Departments of Chemistry and Biochemistry, Widener University, Chester, PA.

Excess nutrients are stored as triglycerides mainly in the adipose tissue of an animal. These triglycerides are located in structures called lipid droplets within adipose cells. Previous genome-wide RNAi screens in *Drosophila* cells identified splicing factors as playing a role in lipid droplet formation. Our lab identified the SR protein, 9G8, as an important factor in fat storage as decreasing its levels results in augmented triglyceride storage in the fat body. However, whether 9G8 interacts with other proteins to affect lipid metabolism is unclear. Previous *in vitro* studies have implicated 9G8 in the control of doublesex (DSX) splicing by binding to transformer (TRA) and transformer2 (TRA2) to regulate sex determination; any function of these proteins in regulating metabolism is unknown. The goal of this study is to determine whether TRA and TRA2 regulate fat storage *in vivo*. To test this hypothesis, we measured triglyceride and glycogen levels in flies with TRA^{dsRNA} or $TRA2^{dsRNA}$ induced in the adult fat body. Decreasing the expression of these sex determination genes resulted in an increase in triglyceride levels, a phenotype similar to the 9G8 knockdown flies. Interestingly, glycogen also accumulated when TRA and TRA2 were decreased in the adult fat body. Consistent with the glycogen and triglyceride phenotypes, TRA and TRA2 knockdown flies lived longer under starvation conditions. In addition, this increase in triglycerides and glycogen is due to more storage of these molecules per cell and not an increase in the number of fat cells as DNA levels are unchanged in TRA^{dsRNA} and $TRA2^{dsRNA}$ flies compared to controls. This triglyceride and glycogen accumulation phenotype also does not seem to be due to more feeding as food consumption was not increased in the TRA^{dsRNA} or $TRA2^{dsRNA}$ flies as measured by CAFÉ assay. We next wanted to determine whether the nutrient storage phenotypes observed here are due to altered expression of the genes coding for important metabolic enzymes. While the levels of major lipid metabolic enzymes were unchanged, the splicing of CPT1, an enzyme involved in the breakdown of lipids, was altered in flies with decreased TRA and TRA2. The less-catalytically active isoform of CPT1 accumulated in TRA^{dsRNA} and $TRA2^{dsRNA}$ flies suggesting a decrease in lipid breakdown, which is consistent with the increased triglyceride levels observed in these flies. Together, these results suggest a link between mRNA splicing, sex determination and lipid metabolism and may provide insight into the mechanisms underlying tissue-specific splicing and nutrient storage in the fat body.

286A Genetics and plasticity of physiological performance in *Drosophila* across development *Kristi Montooth*[1], Luke Hoekstra[2], Cole Julick[1], Omera Matoo[1], Katherine O'Brien[1]. 1) School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE; 2) Department of Evolution, Ecology and Organismal Biology, Iowa State University, Ames, IA.

Development generates a dynamic internal environment that is predicted to impact the fitness effects of mutations that arise in populations. Moreover, the external environment in which development occurs can modify the expression of traits within and across life stages. We and others have shown that even fundamental properties of metabolism (e.g., the scaling laws that govern the relationship between metabolic rate and mass) can vary across development and as a function of the developmental environment (Greenlee, Montooth, Helm. 2014 ICB 54:307). For example, I will show that the scaling of metabolic rate as a function of mass in *Drosophila* depends on the developmental thermal environment, that genetic variance in metabolic rate varies across larval development, and that mitochondrial-nuclear genotype can significantly affect larval metabolic rate and development time. This latter genetic effect is itself conditional on the thermal environment, and we have found that interactions between genotype and thermal environment affect metabolic rate plasticity (i.e., the Q_{10} for metabolic rate) (Hoekstra, Siddiq, Montooth. 2013 Genetics 195: 1129). Environmental perturbations of the photoperiod to increase energy demand during larval growth mimic the thermal-dependent effects of mitochondrial-nuclear genotype on development. Our findings across a number of studies support a model whereby the degree of energy demand may generally explain context-dependent effects of genetic variants across external and internal environments (e.g., sex-, tissue- and life stage-specific effects), particularly for genetic variants that impact metabolism. To better understand the underlying mechanisms that govern the development of energetic processes, we have characterized aspects of metabolism and mitochondrial physiology across development for natural *D. melanogaster* genotypes, as well as for mitochondrial-nuclear genotypes that generate energetic inefficiencies. Finally, I will relate these patterns to plasticity in development, survivorship and gene expression in the presence of ethanol, an important ecological challenge and resource for *Drosophila* larvae.

287B Constructing a Graphical Model of the *Drosophila melanogaster* metabolome *Vishal Oza*, Laura Reed. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Understanding metabolic phenotype, defined as the ensemble state of all metabolites in an organism at any point in time, serves as a key integrative data for studying the complex interplay between the genotype and the phenotype. However, characterization of metabolic phenotypes under different conditions in *Drosophila melanogaster* is at present not achievable mainly because of inability to characterize the unknown portion of the metabolome. The current *Drosophila* metabolic pathway databases are either literature based thus biased towards well studied pathways or predicted from annotated genomes (Genome-scale metabolic models). None of these databases capture the dynamics of metabolic pathways nor address the question of unknown metabolites that are often captured in the untargeted metabolomics studies. Therefore, the need of the hour is to develop analysis methods that will help capture the various dynamics of relationships between metabolites as well as help us incorporate the unknown metabolites within the pathway framework.

Here, we demonstrate the use of Probabilistic graphical models, particularly Bayesian networks to identify the network structure from the untargeted metabolomics data. A total of 856 *Drosophila melanogaster* larvae were subjected to untargeted metabolomics analysis using Liquid Chromatography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry at The West Coast Metabolomics Center. Out of 856 total flies, 99 were control, 381 were fed normal diet (ND), and 376 were fed high fat diet (HFD). The untargeted metabolomics approach isolated and identified concentrations of 422 different metabolites, out of which 169 are known metabolites and 253 are unknown metabolites. The dataset obtained was used to construct a Bayesian network based on a scoring function. The analysis was performed using the *bnlearn* package in R.

The network obtained was a directed acyclic graph. The directionality in the graph indicated the conditional dependence of the metabolites. The method was successfully able to elucidate the linear as well as the non-linear relationships between the metabolites, including the known metabolites not present in current pathway databases as well as unknown metabolites. The network obtained can be further refined by incorporating already known metabolite relationships or can be combined with other *Drosophila* metabolomics studies to build a comprehensive picture of *Drosophila melanogaster* metabolome.

288C Functional analysis of the DHR78 nuclear receptor in adults *Sophia A Praggastis*, Stephanie Marxreiter, Carl S Thummel. Dept of Human Genetics, University of Utah, Salt Lake City, UT.

Nuclear receptors act as ligand-regulated transcription factors that play a vital role in growth, development, and metabolic control. The Testicular Receptor subfamily of nuclear receptors, comprised of TR2 and TR4, remains poorly understood. Although *TR2* mutant mice appear normal, *TR4* mutant mice have a shortened lifespan, reduced levels of stored lipids, resistance to diet-induced obesity, and reduced female fertility. *TR2, TR4* double-mutant mice are embryonic lethal, indicating that these paralogs have redundant functions. We are studying the single *Drosophila* member of this nuclear receptor subfamily, DHR78, to gain insights into TR regulation and function. *DHR78* null mutations result in reduced larval size, developmental delays, and larval lethality due to severe tracheal molting defects. These developmental defects can be overcome by using the GAL4/UAS system to express wild-type *DHR78* in the tracheae, allowing us to study DHR78 functions in the context of adult homeostasis.

Here we show that *DHR78* mutant adults have a reduced lifespan and reduced motility. *DHR78* mutant females have reduced triglyceride levels and smaller lipid droplets in their fat bodies, along with reduced fecundity. In addition, DHR78 is expressed in tissues that play critical roles in metabolic regulation, including the fat body, Malpighian tubules, and intestine. Our current studies are focused on defining the molecular mechanisms by which DHR78 maintains adult physiology and metabolic homeostasis.

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289A Closing the Genotype-Phenotype Gap with Metabolomics *Daniel Promislow*[1,2]. 1) Department of Pathology, University of Washington, Seattle, WA; 2) Department of Biology, University of Washington, Seattle, WA.

Over the past decade, genetic advances have made it relatively easy for researchers to carry out genome-wide association (GWA) studies on a very broad range of complex traits in human populations. These studies have enabled scientists to identify a remarkably large number of loci associated with variation in diverse phenotypes. These associations are typically highly statistically significant, but notable for their very small effect size. Variation at genetic markers typically explains 0.1-0.5% of the variance in the trait of interest, and taken together within a single study, all markers combined usually explain only a small fraction of the total variance (the so-called "missing heritability problem"). A notable exception comes from GWA studies of the metabolome, where single nucleotide polymorphisms can account for 10-40% of the variance in individual metabolites or ratios of two metabolites. Here we present results from studies in *Drosophila* in which we explore the power of the metabolome to link genotype to phenotype.

290B Unique Lifestyle and Metabolism of *Drosophila lutzii*, a Floridosa Species Group Fly
Juan Riesgo-Escovar, Juan Manuel Murillo-Maldonado. Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, México.

At the beginning of the twentieth century Alfred Sturtevant taxonomically described *Drosophila lutzii*, a Neotropical Phloridosa species group of *Drosophila*. Since then, very little work has been done on this species. As its species group implies, they are not saprophytic, but rather feed on flowers. We have found *D. lutzii* eggs, larvae, pupae, and adults inside *Ipomoea sp.* flowers. This suggested a restricted diet, and thus, an interesting avenue for research in metabolism, in comparison to generalist (saprophytic) types of flies. We have found that fresh caught *D. lutzii* have the same amount of carbohydrates as saprophytic species like wild *D. melanogaster* and *D. simulans*, with whom they coexist in the same habitat, but in a different ecological niche. Consistent with a restricted diet and specialist lifestyle, they are less capable of surviving in culture in diets with differing amounts of carbohydrates, and when fed high amounts of sugar, contrary to *D. melanogaster* or *D. simulans*, they significantly accumulate them. They are also significantly and dramatically less motile, but possess a circadian activity rhythm akin to *D. melanogaster* or *D. simulans*. Their metabolic index is also reduced. Overall, they tend to conform to a 'couch potato' type of lifestyle, with little motility, significantly accumulating carbohydrates when fed in 'excess', and to die rather quickly when fed low carbohydrate diets, being capable only of very limited metabolic adjustments.

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291C The microRNA *miR-33* is a regulator of lipid homeostasis in *D. melanogaster*
Dan Fu Ruan[1], Ronald Yu[1], Laure-Alix Clerbaux[2], Guido Bommer[2], Jennifer Kennell[1]. 1) Biology, Vassar College, Poughkeepsie, NY; 2) Institut de Duve, UCL, Brussels, Belgium.

In mammals, SREBPs play a major role in driving expression of genes required for fatty acid and sterol synthesis. Interestingly, the *SREBP* loci not only code for proteins but also for members of the *miR*-33 family. Several studies have demonstrated that *miR-33a* in mammals can cooperate with SREBP2 to maintain cellular lipid levels by reducing cellular cholesterol export and reducing fatty acid degradation. Even though *Drosophila* are auxotrophic for cholesterol, SREBP still plays an important role in flies by promoting expression of enzymes involved in saturated fatty acid synthesis. Our study seeks to study whether *miR-33* might be cooperating with SREBP to regulate lipid synthesis in flies as well. Previous studies have shown that fly *miR-33* can target *cpt1* transcripts and consequently reduce β-oxidation in starved larvae when overexpressed in the fat body. Using cell culture based reporter assays, we have found that, surprisingly, *miR-33* can also directly target mRNAs in *Drosophila* that encode enzymes that are also necessary for fatty acid synthesis and triglyceride synthesis (e.g. ATPCL and Mdy) suggesting *miR-33* may promote free fatty acid levels by reducing both β-oxidation of fatty acids and incorporation of fatty acids into triglycerides. To examine the role of *miR-33* in lipid homeostasis in vivo we generated mutant flies. We hypothesized that *miR-33* mutant flies would have an altered lipid profile, with decreased products of free fatty acids, including phospholipids due to increased shunting of fatty acids to β-oxidation and triglyceride synthesis. Indeed we have found that loss of *miR-33* causes an incompletely penetrant increase in steady state triglyceride levels in adults. The increase in triglycerides is strengthened by removing one copy of the *cpt1* gene and rearing flies on a nutrient deprivation diet. This finding is consistent with the model that loss of some *cpt1* expression in *miR-33* mutants tips the balance towards increased triglyceride synthesis over β-oxidation. We are currently analyzing the complete lipid profile of *miR-33* mutants but our preliminary findings suggest that the *Drosophila miR-33* is likely cooperating with its host gene, *SREBP*, to increase fatty acid levels and thus may indirectly promote phospholipid biosynthesis.

292A MicroRNAs controling body fat content
J. Seo, D. Allen, M. Elledge, R. Arellanes, J. Yeahquo, A. Reiner, S. Zhang, L. Redmond, A. Cotney, E. Espinosa. Department of Biology, Rogers State University, Claremore, OK.

Obesity is closely linked to serious medical conditions such as cardiovascular disease and diabetes; furthermore, obesity is often associated to negative social and emotional impacts including discrimination, lower quality of life, and depression. Thus, both early identification of risk factors and effective therapeutics are crucial to prevent and fight the worldwide epidemic of obesity. MicroRNAs (miRNAs) are small non-coding RNAs; they often regulate cell fate and various developmental processes. Numerous miRNAs have been linked to lipid metabolism, insulin signaling, and adipose tissue development from fruit flies to humans. We screened an *in vivo* UAS-miRNA transgenic library to identify miRNAs regulating total body fat content. Flies containing UAS-miRNAs were crossed to ubiquitously-expressing Actin5C-Gal4 driver line. The F1 adult flies were collected right after eclosion for three days and incubated for another seven days before analysis. Then, the flies were homogenized, and their total body fat contents were measured using a colorimetric triglyceride assay. Wild type flies (W^{1118}) crossed with the same Gal4 driver were used as a base line fat content. We identified multiple miRNAs which alter *in vivo* body fat contents

above 150 % or below 50% comparing to the base line fat content (100%). Since the genes that control fundamental metabolism are well conserved, these findings could be applied to fight current obesity epidemic.

293B The *Drosophila* HNF4 nuclear receptor is required in the oenocytes for cuticular integrity and adult survival *G. Storelli*, C. Thummel. Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

The vertebrate nuclear receptor Hepatocyte Nuclear Factor 4α (HNF4α) regulates a wide range of physiological pathways, including metabolic homeostasis, growth, development, and inflammation. In spite of numerous studies, however, little is known about the molecular mechanisms by which it contributes to these responses. We are studying the *Drosophila HNF4α* ortholog, *dHNF4*, with the goal of defining its regulation and function. Our earlier characterization of *dHNF4* null mutants revealed a critical role for the receptor in lipid and glucose catabolism as well as glucose-stimulated DILP secretion. In addition, *dHNF4* mutants display phenotypes that are still unexplained, including a major lethal phase at adult eclosion, accompanied by reduced adult lifespan, poor fecundity, and inflammation. Interestingly, these phenotypes are also associated with a loss of *HNF4α* function in vertebrates, suggesting that they reflect activities conserved through evolution.

Detailed examination revealed that *dHNF4* mutants tend to stay attached to the pupal case by the tip of their limbs during eclosion, while those that emerge die before cuticle tanning and wing expansion. In addition, *dHNF4* mutants are sensitive to desiccation and survival into adulthood can be rescued by allowing *dHNF4* mutants to eclose in a humid environment. These phenotypes are suggestive of impaired cuticular function, which could arise from roles for *dHNF4* in oenocytes. Consistent with this, oenocyte-specific RNAi for *dHNF4* is sufficient to result in desiccation sensitivity, while oenocyte-specific rescue of *dHNF4* mutants can restore adult survival in dry conditions. In addition, our previous transcriptomic analysis revealed that the expression of genes involved in oenocyte-specific lipid metabolism are reduced in *dHNF4* mutants. Finally, this role for *dHNF4* in oenocytes could explain other phenotypes observed in *dHNF4* mutants, including the inflammatory response and reduced lifespan. Taken together, our studies provide new opportunities to understand the molecular mechanisms by which the HNF4 nuclear receptor regulates lipid metabolism and suppresses inflammation.

This research was supported by a grant from the NIH (DK075607).

294C Biochemical and physiological analyses of *Drosophila* fat body and heart during overnutrition *Bryon F. Tuthill*, Eileen O'Hara, Laura Palanker Musselman. Binghamton University, Binghamton, NY.

Diets high in carbohydrates are associated with metabolic diseases including type 2 diabetes, obesity and cardiovascular disease. Using *Drosophila* as a model organism, a high-sugar diet is employed to study the pathophysiology of these metabolic disorders. We hypothesize that lipid storage in the fat body reaches a maximum capacity, resulting in the accumulation of toxic lipids in other tissues, or lipotoxicity. We are taking tissue-specific genetic and metabolomic approaches to understand lipotoxicity in the fly fat body and heart. Our strategy is to overload the heart by exceeding the storage capacity of the fat body resultant from caloric overload. Preliminary data has shown we can reduce triglycerides when Stearoyl CoA desaturase 1 (desat1) or Carbohydrate Response Element Binding Protein (ChREBP) is knocked down in fat body, compared to wild-type controls. We are currently looking for evidence of cardiac dysfunction in these flies after high-sugar feeding. Combining assays of heart function, morphology, and exercise tolerance during lipid overload with lipid mass spectrometry will allow us to identify lipid metabolic pathways that contribute to fat body overflow and cardiac dysfunction as well as key lipotoxins that contribute to metabolic syndrome. The ultimate goal is to further elucidate the endocrine mechanisms and molecular targets involved in metabolic disease.

295A *Mondo*/*dChREBP* functions in the Dsk neurons to regulate nutrient storage in *Drosophila* *Niahz Wince*, Tyler Avery, Justin DiAngelo. Division of Science, Penn State Berks, Reading, PA.

After a meal, multiple organ systems work together to sense and store nutrients. For example, the brain is known to detect the presence of nutrients and signal liver and adipose tissues to store the energy derived from these nutrients in the form of triglycerides and glycogen. However, the full complement of proteins that function in the brain and the specific neurons in which they act are unknown. Our lab has previously identified the gene *Mondo* as having a metabolic function in the fly brain. *Mondo* is the *Drosophila* homolog of carbohydrate response element binding protein (ChREBP), a transcription factor that stimulates triglyceride storage in the liver in response to elevated nutrient levels. Our lab has shown that *Mondo* functions in the fly brain to control triglyceride and glycogen storage, but the specific populations of neurons in which *Mondo* acts is unknown. To identify neurons in which *Mondo* functions, Mondo-RNAi was induced in several groups of peptidergic neurons including those that produce pigment dispersing factor (Pdf), drosulfakinin (Dsk), neuropeptide-F (NPF), and the insulin-producing cells

(Ilp). Triglyceride and glycogen levels were then measured in these flies with neuronal *Mondo* knockdown. While there was no change in glycogen and triglycerides when *Mondo* was decreased in the Pdf, NPF, or Ilp neurons, we observed an increase in triglyceride storage when Mondo-RNAi was induced in the Dsk neurons. However, in contrast to when *Mondo* is decreased in all neurons, glycogen levels are normal in Dsk-specific Mondo-RNAi flies suggesting that Mondo acts in Dsk neurons to control triglyceride storage but functions in different neurons to control glycogen accumulation. To determine if this elevated triglyceride phenotype was due to an increase in feeding, we measured food consumption over a 24-hour period using CAFE assays. Interestingly, food consumption was similar between control and *Mondo*-knockdown flies suggesting that the triglyceride storage phenotype is not due to increased feeding. Together, these data indicate that *Mondo* functions in the Dsk neurons to regulate organismal triglyceride storage and may act as a sensor to coordinate metabolism with nutrient availability.

296B Microbial quantity contributes to *Drosophila* nutrition. *Erin S. Keebaugh*, Ryuichi Yamada, Kimberley D. Bruce, Sonali A. Deshpande, William W. Ja. The Scripps Research Institute, Jupiter, FL.

In *Drosophila,* microbial association can have beneficial or detrimental effects on host health. Previous studies have focused on identifying the specific microbial strains mediating these effects, and on characterizing changes to enteric microbe composition with host diet and age. Here, we show that the quantity of microbes that flies are associated with, which varies across microbial strains and is influenced by nutritional environment, is a strong predictor of larval development and fly longevity. These effects are replicated by dietary supplementation with a variety of live microbes, heat-killed microbes, or pure protein, suggesting that microbes serve broadly as a source of protein. Because an unanticipated range of microbial species influence host physiology, our results suggest that microbial growth rate and quantity, as influenced by nutritional environment, are the primary drivers for effects on host development and lifespan. Our research on the *Drosophila*-microbe model highlights the complex interaction between microbes and host nutrition, and details how the impact of microbial association is modulated by environment.

297C Localization of histamine to specific regions of the gut in *Drosophila melanogaster*. *Daniel Beachnau*[1], Joel Bonilla[1], Monica Price[1], Kelly Tekiela[2], Martin Burg[1,2]. 1) Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Cell and Molecular Biology, Grand Valley State University, Allendale, MI.

Histamine has been shown to be involved in a number of behavior-related functions in *Drosophila*, such as vision (1), grooming behavior (1), and sleep (2). In these studies, histamine localization to central and peripheral neural tissues was correlated with its presumed function in those tissues, such as histamine localization to photoreceptors and sensory neurons underlying machrochaetae (1). In vertebrates, histamine functions both as a central neurotransmitter and paracrine factor in regulating numerous functions. In particular, the role of histamine in regulating acid secretion in the gut has been well characterized (3). However, a correlate function for gut histamine in *Drosophila* is yet to be identified. We therefore investigated whether histamine could be localized to other tissues of the fly, the gut being our focal point. In larvae, we have detected histamine in at least 4 distinct regions of the gut, by comparing histamine immunolocalization between wild-type flies and histamine-lacking Hdc^{JK910} mutant flies (as a negative control). Therefore, histamine detected in the gut is dependent on the presence of *Hdc* activity in the fly, suggesting that histamine synthesis may be occurring locally. By performing histamine immunostaining on a number of Gal4 lines that drive expression of GFP in either a region- or cell-type specific manner (4), we are identifying the cell types and regions of the gut where histamine is located and *Hdc* expression may be occurring. Confirming the location of histamine to specific regions and cell types of the gut, whose function may be well understood, will provide for a better understanding of the role that histamine may play in the digestive physiology of *Drosophila melanogaster* and potentially other invertebrate species.

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298A An Anti-Stress Command Peptide Regulating Reproduction in *Drosophila melanogaster* *Matthew Meiselman*[1], Michael Adams[1,2]. 1) Department of Entomology, University of California-Riverside, Riverside, CA; 2) Department of Cell Biology and Neuroscience, University of California-Riverside, Riverside, CA.

Ecdysis triggering hormone (ETH) is a command peptide discovered for its role in stimulating the ecdysis behavior, or shedding of the old cuticle, at the end of each molt. After the developmental stages, the gland and peptide persist, raising questions about a secondary role. Here we show that ETH acts as a prolific liberin during the adult stage, regulating octopamine release in the vicinity of the female reproductive tract. In addition to its role in as a regulator of juvenile hormone release, ETH controls a signaling system facilitating not only the synthesis, but the release of nascent oocytes. Additionally, we show that ETH levels

drop during heat and nutritional stress, and that ETH deficiency is critical for stress-induced decrease in fecundity.

299B Roles of *Drosophila* Lipin in the Control of Normal Development *Xeniya V. Rudolf*, Emily Overman, Stephanie E. Greene, Michael Lehmann. Dept Biological Sci, University of Arkansas, Fayetteville, AR.

Proteins of the lipin family are essential in the control of fat tissue development, fat storage and energy homeostasis. Biochemically, lipins have dual functions, acting as both metabolic enzymes in the cytoplasm and as transcriptional regulators in the cell nucleus. We have shown that the single Lipin ortholog of *Drosophila* acts very similar to the three mammalian lipin paralogs. The enzymatic phosphatidate phosphatase (PAP) function of Lipin, which converts phosphatidic acid into diacylglycerol, is required for the production of neutral storage fats (triacylglycerides). Lipin's nuclear functions are negatively controlled by nutrient-sensitive TOR signaling. They are important in low nutrient conditions under which they regulate metabolic genes to promote survival. Interestingly, *Drosophila Lipin* mutants are developmentally delayed, although they grow to normal size, and Lipin is strongly expressed in the prothoracic gland (PG) cells of the endocrine ring gland. Moreover, we found that animals expressing a dominant-negative form of Lipin in the PG cells that lacks the nuclear localization signal (LipinΔNLS) show a severe developmental delay. Together, these data suggest that Lipin may have a role in the production and/or secretion of the molting hormone ecdysone that is produced by the PG cells. Experiments to test this hypothesis are under way. The isolation of *Lipin* mutants that specifically lack either the PAP or nuclear activity will help test this hypothesis and provide crucial information about the biological importance of these two activities. We were successful in isolating a *LipinΔPAP* mutant, which shows early larval lethality, indicating that PAP activity is essential for survival. However, despite repeated and ongoing attempts, we have been unable so far to isolate a *LipinΔNLS* mutant. Since LipinΔNLS expressed from a transgene exerts a dominant-negative effect, we suspect that dominant interference with an essential nuclear function of Lipin might be the reason for this result. This interpretation is consistent with the observed disruption of normal development by expression of LipinΔNLS in the PG cells.

300C Juvenile hormone mimic methoprene regulates the ecdysone response by modifying Ecdysone Receptor function *Rebecca Spokony*[1], Robert Arthur[2], Christopher Brown[3], Kenneth Barr[2], Marium Sarder[1], Jennifer Moran[2], Nicholas Bild[2], Jennifer Zieba[4], Jesse Cohen[2], Kevin White[2]. 1) Natural Sciences, Baruch College, New York, NY; 2) IGSB, University of Chicago, Chicago, IL; 3) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 4) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Drosophila melanogaster development is controlled by two main hormones, ecdysone and juvenile hormone (JH). Ecdysone controls developmental transitions and acts primarily through a heterodimer of two nuclear receptors, Ecdysone Receptor (EcR) and Ultraspiracle (USP). Juvenile hormone controls the type of transitions and acts through two bHLH proteins, Methoprene-tolerant (MET) and Germ cell-expressed (gce). In the presence of JH, MET has been shown to bind Taiman (TAI) and FTZ-F1, two nuclear receptors also involved in the ecdysone pathway. Ectopic juvenile hormone and its mimics can disrupt normal ecdysone mediated processes and is used as an insecticide. Known ecdysone mediated process that are disrupted include polytene puffing and morphogenesis of the central nervous system and salivary glands. *D. melanogaster* is most sensitive to ectopic juvenile hormone at the larval to prepupal transition (pupariation). We exposed white prepupae (WPP) to the juvenile hormone mimic, methoprene at WPP for 4-5 hours. Using ChIP-seq, we characterized EcR binding sites in the absence and presence of methoprene. Using 100 bp windows, there are 186 locations with 4-fold more EcR binding after exposure to methoprene and 825 locations with 4-fold less EcR binding after exposure to methoprene. Differential binding was found at many JH targets' loci, such as Eip75B, ftz-f1, Kr-h1, and tai, as well as differentially expressed genes under ectopic JH or methoprene exposure. Since JHM has been shown to reduce EcR expression, we characterized the JHM sensitivity of 5 putative enhancers bound by **more** EcR after treatment. All of the enhancers drove reporter expression in a tissue specific, JHM-sensitive way. Additionally, expression changes after mutating the EcR binding motif(s) in each enhancer. In 4/5 cases, JHM sensitivity is lost or reversed after EcR binding motif mutation. We will further explore the role of EcR at these enhancers by examining reporter expression after EcR knockdown with RNAi.

301A The Effect of Gut Microiota on Starvation Resistance. *Rachel Hughes*, Alec Judd, Makay White, Madeline Veloz, Lindy Koyle, Corinne Penrod, John Chaston. Brigham Young University, Provo , UT.

Animal-associated microorganisms ('microbiota') have substantial influence on the nutrition and physiology of their hosts, and the fruit fly *Drosophila melanogaster* is an established model to study these interactions. To better understand how the microbiota influence traits related to nutrition, we focus our attention on the influence of the microbiota on fruit fly starvation resistance. In addition to known influence of *Drosophila* genotype on starvation resistance, we show here that on the nutrient-rich diet, bacterial presence substantially shortens the survival of *D. melanogaster* under starvation conditions. To explore the bacterial genetic basis for these effects we conducted a

metagenome-wide association (MGWA) screen. We mono-associated a single CantonS genotype with each of 42 genome-sequenced bacterial strains and measured the influence of each strain on the fly's survival under starvation conditions every 8 hours. Different bacterial strains conferred different starvation resistance on the flies, attributable at least in part to 425 genes that were significantly associated with higher or lower starvation resistance ($p < 1e-9$), including genes involved in vitamin B12 biosynthesis. To confirm the MGWA prediction we obtained vitamin B12 mutants in *E. coli* (cobW) and *Acetobacter fabarum* (cobA), and confirmed that these mutations substantially alter the starvation resistance of their associated flies relative to flies bearing wild-type control strains. We are currently conducting metabolomic analyses to test if these bacterial mutants lower the vitamin B12 assimilation of their host. Together these findings suggest a key role for bacterial vitamin B12 synthesis in the stress resistance and survival of *D. melanogaster.*

302B Mechanisms Regulating Tolerance to Oxidative Stress in *Drosophila melanogaster* *Dan Zhou*[1], Helen Zhao[1], Jin Xue[1], Yu-hsin Hsiao[1], Nitin Udpa[2], Vineet Bafna[2], Gabriel Haddad[1,3,4]. 1) Dept Pediatrics, Univ Califonia, San Diego, La Jolla, CA 92093-0735; 2) Department of Computer Science and Engineering, University of California San Diego, La Jolla, CA 92092; 3) Department of Neuroscience, University of California San Diego, La Jolla, CA 92093; 4) The Rady Children's Hospital, San Diego, CA 92123.

Oxidative injury is a common condition seeing in many diseases, including neurodegenerative diseases, which may lead to serious health consequences in children and adults. In order to dissect mechanisms protecting cells again oxidative injury, we generated a unique *Drosophila melanogaster* strain that can tolerate severe, normally lethal, hyperoxic conditions through laboratory selection with gradually elevated oxygen levels (i.e., the hyperoxia-selected flies (HOF)). By comparing naïve control and HOF flies, we have identified differences in genomic DNA sequences as well as gene and protein expression that regulate response to oxidative injury. Importantly, we have confirmed experimentally the role of a subset of these candidate genes in protecting against oxidative injury. Interestingly, the majority of the differentially expressed mitochondrial proteins were found to be the modifiers of Notch signaling. Furthermore, we found that the *kst* gene is under strong selection in the HOF populations ($p<0.01$, McDonald-Kreitman test), and its expression was significantly up-regulated in the HOF flies. We believe that the Notch signaling and the *kst* gene play an important role in regulating cellular tolerance to oxidative stress in flies.

303C Mitonuclear interactions alter the impact of diet restriction on starvation resistance, and aging in Drosophila *B. Franklin*, J. Santiago, F. Lemieux, J. Mossman, D. Rand. Dept. of Ecology & Evolutionary Biology, Brown University, Providence, Rhode Island.

Effective communication and cooperation between the genes encoded in the nuclear and mitochondrial genomes are essential in regulating central metabolism and energy production. Breakdown in these mitonuclear interactions can lead to a variety of disease and reduce lifespan. To model this process, we placed alternative mtDNA genotypes from *D. melanogaster* and *D. simulans* on to different nuclear genomic backgrounds from the Drosophila Genetic Reference Panel (DGRP) and quantified the joint impact of mtDNA genotype, nuclear genotype and protein- and sugar-restriction diets on starvation resistance, longevity and biochemical composition. Older flies show reduced starvation resistance but this effect is strongly dependent on mtDNA x nuclear genotype combinations. Clear interactions between specific DGRP nuclear genotypes and mtDNAs alter starvation resistance and longevity on high-protein vs. high-sugar diets. We quantified levels of lipids, protein, and carbohydrates under different diet conditions and found significant shifts in composition across specific pairs of DGRP genotypes carrying alternative mtDNAs. These mito x nuclear x diet interactions are distinct enough to allow the mapping of nuclear loci that modify mitochondrial function. 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).

304A Regulation of mitochondrial function by the Condensin II complex Emily Deutschman[1,2], Kavitha Sarvepalli[1], Anny Mulya[3], Madeleine Lemieux[4], John Kirwan[3], *Michelle Longworth*[1]. 1) Cellular and Molecular Medicine, Cleveland Clinic Lerner Res Inst, Cleveland, OH; 2) Genetics and Genome Sciences, Case Western Reserve University, Cleveland OH 44145; 3) Department of Pathobiology, Cleveland Clinic Lerner Res Inst, Cleveland OH 44195; 4) Bioinfo, CA, Plantagenet ON, Canada.

Mitochondrial function is important for ATP synthesis and its regulation is therefore paramount to maintaining cellular homeostasis. The Condensin II complex is involved in regulating genome organization and gene transcription throughout the cell cycle. While Condensin II-mediated processes in the nucleus are well-studied, the function of cytoplasmic Condensin II complexes remain unknown. Through RNA-sequencing studies we have determined that depletion of dCAP-D3 *in vivo* in multiple organs of *Drosophila* larvae results in the upregulation of mitochondrial-encoded genes. This is accompanied by mitochondrial hyperactivity, as measured by Tetramethylrhodamine, Ethyl Ester, Perchlorate (TMRE) incorporation and increased generation of reactive oxygen species. Our data suggest that Condensin II may be regulating mitochondrial encoded gene transcription and respiratory function directly at the site of the organelle, as immunofluorescence analysis demonstrates that dCAP-D3 and another Condensin II subunit,

dCAP-H2 both localize to mitochondria.   Interestingly, dCAP-D3 depletion also results in increased mitochondrial genome copy numbers, and we are actively investigating the role that Condensin II might play in organizing the mitochondrial genome, in regulating mitochondrial DNA replication, and in controlling mitochondrial biogenesis. Excitingly, human CAP-D3's role in regulating mitochondrial function appears to be conserved, as human colon adenocarcinoma (HT-29) cells also exhibit mitochondrial dysfunction following a reduction in CAP-D3 levels.

305B   Sexual Asymmetry in dTOR Regulation of Mitochondria   *John C Santiago*[1], Cynthia Hale-Phillips[2], Brian Franklin[3], David Rand[1,3]. 1) Molecular Biology, Cellular Biology and Biochemistry, Brown University, Providence, RI; 2) Department of Engineering, Biomedical Engineering, Brown University, Providence, RI; 3) Department of Ecology and Evolutionary Biology, Brown University, Providence, RI.

Rapamycin has been shown to extend lifespan by potently inhibiting TOR (Target of Rapamycin) signaling, a major component of cellular metabolism. One fundamental yet poorly understood role of TOR is to modulate mitochondrial function. Previously we have shown that rapamycin enhances mitochondrial respiration in female flies, a beneficial effect that is eliminated in flies carrying a foreign mtDNA on the same D. melanogaster chromosomal background (Villa-Cuesta et al. 2014). Here we show a gender specific role of TOR signaling where rapamycin treatment increases mitochondrial oxygen consumption in female Drosophila but not in males. Interestingly, in D. melanogaster males carrying foreign mtDNA rapamycin increases mitochondrial respiration, unlike the effect in females. To better understand this sexually dimorphic response we utilized gene-switch induced RNAi knockdowns of the TOR-complex (TORC1) components Raptor and TOR. We test the hypothesis that genetic disruption of TORC1 will induce an effect similar to that seen in response to rapamycin treatment. Disruption of TORC1 in both females and males with either TOR or Raptor RNAi increased mitochondrial respiration. These results support a novel model in which this sexually dimorphic rapamycin response is not due to the absence of TORC1 signaling but to a sex specific function of rapamycin. Ongoing studies will examine the effects of alternative mtDNAs paired with RNAi against TOR, Raptor and Rictor to dissect the roles TORC1 and TORC2 in mitochondrial function. This model system provides novel approach to dissecting the gender-specific components of TOR signaling.

306C   Epigenetic effects of aging on the germline of *Drosophila melanogaster*   *Alexandra Erwin*, Justin Blumenstiel. University of Kansas, Lawrence, KS.

Epigenetic changes have been associated with the aging soma, but little is known about epigenetic changes in the germline and other reproductive tissues during aging. We analyzed RNA-seq data from aged stage 14 egg chambers from *Drosophila melanogaster* females. Stage 14 represents the last stage of oogenesis before fertilization and oviposition. By focusing on this stage of development, we can investigate how aging jointly effects both the soma (follicle cells) and germline (oocyte) of developing eggs.

We find that both germline and somatic transcripts are altered across age in both strains. Most notably, transcripts of the mitochondrial genome as well as nuclear genes involved in mitochondrial organization and oxidation processes have altered transcription in eggs of aged mothers. We also find enrichment of differentially expressed genes on the heterochromatic 4th chromosome of *D. melanogaster*.

Numerous studies have shown transposable element (TE) de-repression in the aging soma, thought to be a result of heterochromatin redistribution. We asked if this de-repression of TEs also occurs in aged reproductive tissues. We found one retrovirus-like element, *copia*, that increased during aging in both Drosophila melanogaster lines that we analyzed. There were several other TEs that showed an age effect on expression, but only in one strain. Additionally, these TEs showed a decrease in aging as opposed to the age-induced increased expression found in other studies. Thus, global TE de-repression in aging tissue may be exclusive to the soma and depend on both the genetic background and the TE family.

To tease apart aging affects between the soma and germline, we sequences transcripts of 0-1 hour embryos from young and old mothers. Because embryos at these time-points have not yet begun zygotic transcription, this allows us to evaluate the affect of age on germline transcripts. We find that some transcripts show the same patterns between both embryos of young and old mothers as well as egg chambers, while other age-related transcript changes are driven primarily by the somatic follicle cells. While one transposable element also shows a significant change in expression between embryos of young and old mothers, there is no evidence of a global TE de-repression in the germlines of young and old flies. This study offers unique insight into the epigenetic effects of aging on the germline.

307A   Gut Bacteria Supplements – Best Frenemies?   *Kaitlyn Grayson*, Nicole Bournias-Vardiabasis. Biology, California State University, San Bernardino, Claremont, CA.

Microbiota in the gut have a symbiotic relationship with their host organisms. Some aid in digestion and the breakdown of nutrients and others can help regulate intestinal physiology. However, others may not be as beneficial and feed on the epithelial lining of the gut, which can lead to inflammation. This field of study is relatively new and the effects of microbiota are not fully understood. In *Drosophila*, previous studies have shown a relatively low

diversity in the fruit fly's gut. In order to further our current understanding, it was decided to use probiotic cultures and alter bacterial ratios to identify the effects that various species may have on overall fly health. For this study, two strains of *Drosophila melanogaster* were used: Dcy KO and Dcy rescue. Dcy KO flies are a strain that is unable to produce the Dcy protein, which is an important structural component of the peritrophic matrix. Due to this deficiency, Dcy KO flies have a leaky gut, which makes them more susceptible to inflammatory disease. Dcy rescue flies served as the control group. Within these two main groups, there were four subgroups (depending on the microbes used) with 75 male individuals each: control, *A. fumigatus*, *S. marcescens*, and *L. plantarum*. The control group were fed standard cornmeal-sucrose medium. The other three were provided medium inoculated with 50 µL of bacterial or fungal suspension. Medium was changed once per week to maintain freshness. The bacterial and fungal solutions had 50 µL of the broth extracted then the 50 µL extracted placed in new broth to maintain a healthy population every two weeks. A lifespan observation was performed and fly populations were checked once a week. The lifespan observation indicated that *S. marcescens* reduced the lifespans of both genotypes by an average of one week. *A. fumigatus* exposure resulted in a very slight decrease in the Dcy KO populations' lifespans but it was not statistically significant. *L. plantarum* exposure didn't have any significant effect. Heightening the insects' exposure to *L. plantarum* probably did not have a significant effect because this is the predominant species found within *Drosophila*'s gut microbiome and it simply filled up other niches while continuing regular functions. *A. fumigatus* most likely didn't have a significant effect because yeast cells are larger than bacterial cells and therefore could not penetrate the peritrophic matrix. Future studies will include the addition of a nutritional supplement to determine if the altered bacterial ratios affect nutrient absorption. In addition, a courtship assay will be performed to further understanding on how changes in the microbiome affect behavior patterns.

308B Neuronal expression of an evolutionary conserved metallophosphodiesterase regulates *Drosophila* lifespan. *K. Gupta*[1], V. Janardan[1], D. Mahishi[1], R. Padinjat[2], S. Visweswariah[1]. 1) Department of Molecular Reproduction,Development and Genetics, Indian Institute of Science, Bangalore, India; 2) National Centre for Biological Sciences, Bangalore, India.

Rv0805 (a novel class III mycobacterial metallophosphoesterase) was characterised biochemically and structurally in our laboratory. Bioinformatic analysis identified mammalian orthologs of Rv0805 annotated as MPPED1 and MPPED2 (Metallophosphoesterase Domain Containing Protein 1 and 2). In humans, the *mpped2* gene is located on chromosome 11 in a locus associated with WAGR syndrome, suggesting its role in neuronal development and function. We chose *Drosophila melanogaster* as our model system to understand the biological role of the MPPED1/MPPED2 family of proteins. *Drosophila melanogaster* harbours a single ortholog of MPPED1/MPPED2 family annotated as CG16717 (dMPPED1). *In vitro* biochemical analysis suggested that dMPPED1 is a metallophosphodiesterase. dMPPED1 transcripts were found to be expressed throughout the fly lifecycle and expressed in multiple tissues in the adult, with maximal expression in the brain. We generated a targeted knockout of the *dmpped1* gene using homologous recombination. dMPPED1^{KO} flies were homozygous viable. However, dMPPED1^{KO} flies exhibited reduced median lifespan in comparison to wild type flies, and life span was restored following neuronal expression of dMPPED1. Neuronal expression of MPPED2 was also able to restore the reduced lifespan of dMPPED1^{KO} flies, suggesting the functional conservation among the two proteins. Interestingly, over-expression of dMPPED1 in wild-type flies led to an increase in their lifespan. To elucidate the mechanisms by which dMPPED1 regulates fly lifespan, dMPPED1^{KO} and wild type flies were subjected to dietary restriction. Both dMPPED1^{KO} and wild type flies showed an extension in lifespan upon dietary restriction, and this coupled with similar expression of IIS and TOR pathway transcripts between wild-type and dMPPED1^{KO} flies indicated that dMPPED1 regulates lifespan independent of insulin/TOR pathways. Future studies will be directed to elucidate the mechanisms by which dMPPED1 regulates *Drosophila* lifespan.

309C Proteasome Subunit Overexpression Reduces Protein Aggregates and Extends Lifespan Nga Nguyen[1], Anil Rana[2], David Walker[2], *Jae Hur*[1]. 1) Dept of Biology, Harvey Mudd College, Claremont, CA; 2) Dept of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA.

The accumulation of oxidative damage to proteins results in unwanted covalent modifications that, over time, can result in detrimental protein misfolding and aggregation. Accordingly, the degradation and removal of oxidatively damaged proteins in the cytoplasm by the proteasome, a central part of the ubiquitin proteasome system (UPS), has been shown to play a pivotal role in the maintenance of a functional proteome, and approaches to increase protein turnover by boosting UPS components have been shown to be effective in ameliorating aging associated phenotypes and extending lifespan. Moreover, previous reports have shown that aging is associated with a decline in proteasome activity which may increase the prevalence of protein aggregates in older flies. Owing to their complexity, wholesale overexpression of the proteasome is technically unfeasible, but studies in other models have implicated some subunits of the proteasome, including the catalytically active β5 subunit, to have regulatory functions. We show that overexpression of the β5 subunit in adult flies results in significantly increased chymotrypsin-like enzymatic activity and extension of lifespan. Moreover, the β5 overexpression mediated lifespan

extension did not overlap with dietary mechanisms, with no decrease in food consumption, weight loss, or significant change to activity. Additionally, we find that while β5 overexpression is not sufficient to significantly alter the expression of other proteasome subunits or alter other proteasome-associated enzymatic activities, it is sufficient to decrease the size and number of ubiquitinated protein aggregates in aged flies, suggesting that maintenance of β5 and UPS are important for proteome health and organismal longevity.

310A Intestinal Microbes Shorten The Host Lifespan With Increased Intestinal Permeability In Drosophila melanogaster *Hye-Yeon Lee*, Shin-Hae Lee, Woong Seo, Kyung-Jin Min. Inha University, Incheon, South Korea.

Intestinal microbes that live in a symbiotic relationship with their host are well known to affect host fitness such as metabolism, obesity, and inflammation. However, the effects of intestinal microbes on host lifespan are not well characterized. In this study, we investigated the effects and mechanisms of intestinal microbes on host lifespan using the *Drosophila melanogaster* as a powerful model animal to study host-microbes interaction. We generated axenic flies by dechorionation of eggs with sodium hypochlorite, and measured its lifespan. The lifespan of flies was increased under axenic condition, and it was decreased by the oral ingestion of the extracts of guts from female flies, indicating that the microbes existing in the gut of flies affect host lifespan. To investigate which commensal bacteria affect the host lifespan, adult flies was subjected to oral ingestion with the single species of microbes such as *Lactobacillus brevis*, *Acetobacter persici*, *Lactobacillus plantarum* and *Acetobater malorum* which are dominated in the guts of young or old flies. We hypothesized dysbiosis of the intestinal microbiota leads to systemic influences on aging files with increased intestinal permeability. The incidence of intestinal dysfunction was increased by aging and intestinal dysfunction increased the permeability of microbes in the gut of flies. In addition, we interestingly observed that *L. plantarum, Sphingomonas yunnanensis, L. brevis,* and *Acetobacter indonesiensis* were founded in the hemolymph of flies with intestinal barrier dysfunction. Taken together, our findings suggest the possibility that intestinal microbes decrease the host lifespan with increased intestinal permeability by aging.

311B Escargot is Required for Accumulation of Atypical Intestinal Cells Caused by Aging *Charles Choi*[1], D. Leanne Jones[1,2]. 1) Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, CA; 2) Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA.

The *Drosophila* intestinal epithelium is maintained by intestinal stem cells (ISCs) that self renew or differentiate into transitory enteroblasts (EBs) that further differentiate into epithelial cells that carry out the digestive function of the intestine: secretory enteroendocrine cells (EEs) and absorptive enterocytes (EC). ISCs and EBs express the transcription factor Escargot (Esg), a member of the highly conserved Snail family of transcription factors. Esg expression in ISCs and EBs is crucial for stem cell maintenance, and Esg levels influence the differentiation decision between EE and EC cell fates. In aged flies, however, there is an increase in ISC division and an accumulation of *esg*-positive cells that appear to be polyploid, a feature typically restricted to ECs, raising the possibility that *esg* expression is deregulated in aged flies. These cells express additional markers of terminally differentiated ECs, further demonstrating the uniqueness of these cells from intestinal cells of healthy flies. To determine whether persistent *esg* expression has a role in intestinal aging, *esg* was depleted by transgenic RNAi expression using the *esg-GAL4*-driver in aged flies, which resulted in fewer polyploid esg::GFP cells compared to un-induced controls. Furthermore, *esg* RNAi expression driven by *MyoIA-GAL4*, which is normally expressed in differentiated ECs, also reduced the number of polyploid esg::GFP cells. These results suggest that age-induced accumulation of polyploid esg::GFP-positive cells can be reversed by *esg* knockdown. Ongoing experiments will address a requirement for Esg in driving additional aging phenotypes such as increased inflammatory signaling, bacterial dysbiosis, and loss of intestinal barrier function.

312C Two Cytochrome p450s Essential for Peritrophic Matrix Synthesis *Sean Conway*, Edward Blumenthal. Biological Sciences, Marquette University, Milwaukee, WI.

Maintenance of epithelial barriers is essential for an organism's survival as they allow the organism to regulate their interaction with the environment. Mutation in the *Drosophila* gene *drop-dead (drd)* produces flies that exhibit various phenotypes of malfunctioning barrier structures including neurodegeneration leading to early adult lethality, fragile eggshell formation leading to female sterility and the absence of a midgut extracellular barrier called the peritrophic matrix (PM). *drd* is expressed at high levels during stage 10 of oogenesis and a microarray study from stage 10 egg chambers in *drd* knockdowns revealed three gene families with significantly altered expression: cytochrome p450s, juvenile hormone epoxide hydrolases, and the epsilon subfamily of glutathione S transferase. An RNAi screen knocking down genes from these three families in the cardia, the site of PM synthesis, revealed that two cytochrome p450s, *Cyp6a22* and *Cyp6t1*, mirror the *drd* phenotype of lacking a PM. Additionally, d*rd*, *Cyp6a22* and *Cyp6t1* knockdowns exhibit elevated levels of the immune response gene *diptericin* (14 fold increase vs WT in *Cyp6a22* knockdown, 16 fold increase in *Cyp6t1* knockdown vs WT) and lowered rates of defecation (55.7% decrease in *Cyp6a22* knockdown vs WT and 42.3% decrease in *Cyp6t1* knockdown vs WT). To

assess if *Cyp6a22* and *Cyp6t1* may be implicated in additional *drd* pathways, *Cyp6a22* and *Cyp6t1* were knocked down globally. However, unlike *drd*, neither *Cyp6a22* nor *Cyp6t1* global knockdowns exhibit early adult lethality nor female sterility. Therefore, it is possible *Cyp6a22* and *Cyp6t1* are functioning in the same PM synthesis pathway as *drd* but may not have a significant role in the *drd* pathways for egg shell formation and neurodegeneration. This project was supported by NSF grant, IOS-1355087.

313A The effect of male and female genotype on post-mating gut remodeling in female *Drosophila melanogaster* *M.A. White*[1], A. Bonfini[2], A. Clark[1], N. Buchon[2], M.F. Wolfner[1]. 1) Cornell University Department of Molecular Biology and Genetics, Ithaca, NY; 2) Cornell University Department of Entomology, Ithaca, NY.

Females invest large quantities of nutrients into egg development. To cope with the large shift in metabolism that follows mating, females undergo widespread physiological and behavioral changes, including increasing food intake and altering digestive parameters. Recent findings show that the female's primary organ of digestion, the midgut, enlarges significantly after mating, possibly to facilitate greater nutrient absorption and maximal egg production (Reiff et al., 2015, *eLife*). Here, we show that the male seminal fluid protein, Sex Peptide (SP), is required for this post-mating gut enlargement: guts of females mated to males lacking SP do not differ significantly in size from those of virgin females. To further characterize the effects of mating and SP on gut size we are examining effects of mating on different regions of the gut. We also have initiated studies of variation in post-mating effects on gut size across the Drosophila Genetic Reference Panel (DGRP) to determine relative contributions of male cues and female responses in post-mating gut growth and, by GWAS, to identify genes associated with this response.

314B Tissue-specific insulin signaling mediates female sexual appeal *Tatyana Fedina*[1], Devin Arbuthnott[2], Howard Rundle[3], Daniel Promislow[4], Scott Pletcher[1,5]. 1) Department of Molecular and Integrative Physiology, University of Michigan, 109 Zina Pitcher Pl, Ann Arbor, MI 48109, USA; 2) Department of Zoology, University of British Columbia, #4200-6270 University Blvd., Vancouver, B.C., Canada, V6T 1Z4; 3) Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada, K1N 6N5; 4) Department of Pathology, University of Washington, 1959 NE Pacific Street, Box 357705, Seattle WA, 98195, USA; 5) Geriatrics Center, University of Michigan, 109 Zina Pitcher Pl, Ann Arbor, MI 48109, USA.

Individuals must choose their mates "wisely" to maximize reproductive success, and this choice is usually based on specific attractiveness traits. Such traits are assumed to reflect an underlying quality, although sometimes trait value can be faked by less fit individuals. Little is known about why specific traits are chosen for quality assessment and how reliable they are. Identifying the mechanistic connections between attractiveness traits and fitness would provide insight into these issues. Global modulation of insulin signaling (IS) has been shown to influence sexual attractiveness in *Drosophila*. In this study we ask whether the effects of IS on attractiveness derive directly from reproductive tissues, or whether they are more representative of an organismal state reflecting IS activity in multiple tissues. We used transgenic Gal4-UAS system to manipulate IS in adult female tissues, and behavioral assays to measure female attractiveness; ovary size, fecundity and pheromone profiles (GC) were analyzed as well.

We found that IS does not act directly on the pheromone-producing cells (oenocytes); instead it modulates oenocyte activity non-autonomously through its effects in fat body and ovarian follicle cells. Vitellogenic ovaries were required for cell non-autonomous effects to manifest, and IS in late follicle cells was sufficient to recapitulate the potentiation of attractiveness by global IS activation. Interestingly, modulation of IS in the fat body produced effects of the opposite sign, suggesting a sink-source relationship between the ovary and the fat body. Ovary size and fecundity were positively correlated with attractiveness across all relevant IS manipulations, suggesting involvement of this pathway in reliability of fecundity cues. We describe a system in which female attractiveness is determined by an organismal state of high fecundity, which is created by low IS in the fat body and high IS in late follicle cells. This study opens an avenue for future investigation of molecular connections between these tissues, to determine the basis and extent of reliability in Drosophila sexual signaling system.

315C Innate immune signaling in the *Drosophila* larval fat body disrupts nutrient storage *Brittany A. Martinez*[1], Emily Y. Scott[1,2], Michelle L. Bland[1]. 1) Department of Pharmacology, University of Virginia, Charlottesville, VA; 2) School of Pharmacy, Virginia Commonwealth University, Richmond, VA.

Insulin signaling in well-fed animals promotes triglyceride storage, providing the animal with an immense pool of untapped energy when food is unavailable. In flies, signaling through the innate immune Toll pathway inhibits the insulin signaling pathway and shifts metabolism to decrease triglyceride storage, perhaps to reroute energy to the metabolically-costly process of fighting infection. How the crosstalk between insulin and innate immune signaling alters metabolism is not understood. Blocking lipolysis at the lipid droplet through inhibition of the rate-limiting lipase, brummer (*bmm*), an ATGL homolog, does not restore triglycerides in response to Toll activation. This data suggests that not only is an increase in lipolysis not occurring, but also that an increase in β-oxidation is unlikely to underlie the decrease in triglycerides seen with Toll activation. Larvae with active Toll signaling exhibit no change in circulating sugar, which is the substrate for de novo lipogenesis. In multiple rescue experiments, results show that the decrease

in triglycerides seen with Toll activation can be rescued with a constitutively active Akt protein (*myrAkt)*, a major downstream regulator in the insulin signaling pathway. Two major targets of Akt signaling that regulate de novo lipogenesis and triglyceride stores are mTORC1, which activates the transcription factor SREBP1, and the transcription factor FOXO. mTORC1 is under inhibitory regulation by TSC1; however, TSC1 knockdown was unable to rescue the decrease in triglycerides in larvae with active Toll signaling. In contrast, we find that knockdown of FOXO rescues the decrease in triglycerides seen with Toll activation. This suggests that a transcriptional mechanism regulates the triglyceride phenotype. Identification of FOXO-regulated genes in flies with active Toll signaling should shed light on the mechanism accounting for decreased triglycerides in these animals. Finally, flies with decreased triglycerides due to Toll activation have an increased level of glycogen, which serves as another glucose storage pool for energy consumption in fasting animals. This raises the possibility that flies with active Toll signaling could be diverting their glucose to making glycogen instead of triglycerides, thereby reducing ATP consumption for nutrient storage. In summary, a more clear understanding of how the insulin signaling and innate immune signaling pathways regulate metabolite storage will aid in deciphering specific mechanisms of how these pathways work to balance each other, especially in disease states.

316A Activin-Beta/TGF-Beta signaling in skeletal muscle controls insulin/TOR signaling, metabolism, and body size *Lindsay Moss-Taylor*, Michael O'Connor. University of Minnesota, Twin Cities, Minneapolis, MN.

Inter-organ communication is essential for regulating development and homeostasis. Mutations in the gene coding for the Drosophila TGF-Beta ligand *Activin-Beta* (*Act-Beta*) cause accelerated pupariation and reduced final body size. To determine how *Act-Beta* affects size and timing, we first looked at which cells express *Act-Beta* and found expression in the Insulin Producing Cells (IPCs), neuroendocrine cells, and motor neurons. In *Act-Beta* mutants, overexpressing *Act-Beta* in neuroendocrine cells or motor neurons can rescue body size but not developmental timing, indicating *Act-Beta* regulates these processes independently. Accordingly, the growth rate of *Act-Beta* mutants is reduced, demonstrating the size phenotype is not simply due to early growth termination from precocious timing. Muscle-specific knockdown of the TGF-Beta signaling transducer/transcription factor dSmad2 also reduces body size, identifying muscle is a target tissue of the Act-Beta signal. Additionally, levels of phospho-dSmad2 are reduced in skeletal muscle samples of *Act-Beta* mutants and increased in animals overexpressing *Act-Beta* from motor neurons. Levels of phospho-S6K in *Act-Beta* mutants are correlated with phospho-dSmad2 levels, suggesting TGF-Beta signaling regulates insulin/TOR signaling. Because insulin signaling controls metabolism, we used GC/MS analysis to identify and quantify levels of metabolites in whole-larval samples of *Act-Beta* mutants. We found intermediates of the energy-producing steps of glycolysis and lactic acid are reduced in *Act-Beta* mutants. Overall, this indicates neuronally-derived Act-Beta signals to the skeletal muscle to regulate levels of insulin signaling, metabolism and growth to control body size. We have identified over 300 downstream targets of dSmad2 using RNA-seq of *Act-Beta* mutant skeletal muscle and are testing the function of certain target genes in skeletal muscle to determine how Act-Beta/dSmad2 signaling regulates insulin/TOR signaling, metabolism and body size.

317B Fat body insulin signaling and the immune response *Laura Musselman*[1], Tatiana Requijo[1], Jared Gatto[1], Matthew Pereira[1], Thomas Baranski[2]. 1) Biological Sciences, Binghamton University, Binghamton, NY; 2) Washington University School of Medicine, St. Louis, MO.

Insulin resistance in type 2 diabetes is associated with cardiovascular disease, inflammation, blindness, peripheral neuropathy, non-alcoholic fatty liver disease, and obesity. Type 2 diabetics also exhibit a paradoxical increase in inflammation along with an increase in susceptibility to infection. We use a model of diet-induced type 2 diabetes in the fly to dissect the genes and pathways involved in the systemic response to overnutrition. Flies fed high-sugar diets exhibited hyperglycemia, obesity, cardiovascular complications, increased susceptibility to infection, and reduced longevity, similar to human patients who overeat.

Interested in the downstream mediators of insulin signaling that contribute to this range of pathophysiological consequences, we used loss- and gain-of-function genetics to identify differentially-expressed genes downstream of InR. Interestingly, genes encoding proteins reported to function in the response to infection were over-represented in both datasets. Factors involved in protein folding and translation, metabolic enzymes, and novel proteins also seemed to play a role based on differential expression analyses. We have tested the roles of some of these proteins to see whether they affect glucose metabolism and insulin signaling in the fat body. Our studies have identified several genes that connect metabolism to the immune response in our model of diet-induced type 2 diabetes.

318C High fat diet-induced TGF-β/Gbb signaling provokes insulin resistance through the tribbles expression *K Yu*[1,2,3], SH Hong[1], E Yeom[1], KS Lee[1,3]. 1) Neurophysiology & Metabolism Research Group, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea; 2) Convergence Research Centre for

Dementia, Korea Institute of Science and Technology (KIST), Seoul, Korea; 3) Functional Genomics Dept., University of Science and Technology (UST), Daejeon, Korea.

Hyperglycemia, hyperlipidemia, and insulin resistance are hallmarks of obesity-induced type 2 diabetes, which is often caused by a high-fat diet (HFD). However, the molecular mechanisms underlying HFD-induced insulin resistance have not been elucidated in detail. In this study, we established a Drosophila model to investigate the molecular mechanisms of HFD-induced diabetes. HFD model flies recapitulate mammalian diabetic phenotypes including elevated triglyceride and circulating glucose levels, as well as insulin resistance. Expression of glass bottom boat (gbb), a Drosophila homolog of mammalian transforming growth factor-β (TGF-β), is elevated under HFD conditions. Furthermore, overexpression of gbb in the fat body produced obese and insulin-resistant phenotypes similar to those of HFD-fed flies, whereas inhibition of Gbb signaling significantly ameliorated HFD-induced metabolic phenotypes. We also discovered that tribbles, a negative regulator of AKT, is a target gene of Gbb signaling in the fat body. Overexpression of tribbles in flies in the fat body phenocopied the metabolic defects associated with HFD conditions or Gbb overexpression, whereas tribbles knockdown rescued these metabolic phenotypes. These results indicate that HFD-induced TGF-β/Gbb signaling provokes insulin resistance by increasing tribbles expression.

319A Functional study of P450 enzymes controlling development in Drosophila *Ruoying Lu*[1], Nakoki Yamanaka[2]. 1) Biochemistry, University of California, Riverside, Riverside, CA; 2) Entomology, University of California, Riverside, Riverside, CA.

Cytochrome P450s are a group of enzymes present in a variety of organisms and originally characterized as detoxification oxidases. After decades of study, it has been revealed that they are also involved in biosynthetic reactions of steroid hormones in both vertebrates and insects. 20-hydroxyecdysone (20E) plays important roles in *Drosophila* development as the molting hormone promoting molting and metamorphosis. We identified some P450 enzymes whose deficiency causes developmental defects in *Drosophila*. They are evolutionarily close to the 20E biosynthetic enzymes encoded by Halloween genes and highly conserved among insect species. Null-mutants were created by CRISPR/Cas9 and analyzed for developmental defects. Two of the mutants showed lethality as prepupae, and one has defects at eclosion. These phenotypes are similar to some ecdysteroid deficient mutants, indicating that these genes may be involved in the regulation of ecdysone signaling in *Drosophila*. An *in vitro* conversion system using transfected S2 cells was established and optimized for screening of putative substrates of these enzymes.

320B The microbiota affects Alcohol Dehydrogenase protein levels and the response to alcohol *Malachi Blundon*[1], Anna Pyzel[1], Tiffany Lau[1], Jennifer Huang[1], Scott Keith[1], Stacie Oliver[1], Rory Eutsey[1], Jon Cohen[2], Annie Park[3], Nigel Atkinson[3], Luisa Hiller[1], Jon Minden[1], Brooke McCartney[1]. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) School of Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Neuroscience, University of Texas at Austin, Austin, TX.

Symbiotic relationships between microbes and animals are ubiquitous in nature. The animal microbiota is a vast and diverse population of bacteria and other microbes living symbiotically with their hosts that has a profound influence on many aspects of host physiology, including metabolism, immunity, development, and behavior. However, little is known about the molecular crosstalk between microbes and host that mediate these effects, particularly in the area of brain function and behavior. To address this problem, we employed a proteomic technique call Two-dimensional Difference Gel Electrophoresis (2D-DIGE) to examine the protein differences in heads between conventionally-reared (CV) flies and axenic (AX) flies, those raised in a sterile environment. We found that the level of Alcohol Dehydrogenase (ADH), a key enzyme in alcohol metabolism, is elevated in the heads of AX males and females, suggesting that AX flies may have altered physiological and behavioral responses to ethanol (EtOH) exposure.

We found that AX males are significantly less sensitive to the immobilizing effects of EtOH vapor than their CV counterparts. This increased EtOH sensitivity is partially reverted and the ADH protein levels are returned to normal when restoring the Drosophila microbiota to GF flies 0-24 hours after eclosion. This suggests that the microbiota plays an important role in the response of Drosophila to EtOH. We are currently testing whether ADH metabolic activity is necessary for the difference in physiological response to alcohol between CV and AX flies. We are also interested in identifying what taxa of the microbiota is mediating the difference in physiological response to alcohol between CV and AX flies. Finally, we are asking whether AX flies have a difference in their preference for alcohol. Taken together, these data suggest that the physiological responses to alcohol are controlled in part by the microbiota, and these novel insights may lead to an understanding of the microbiota's role in alcohol abuse disorders.

321C A Neuroendocrine Network Connecting Gustatory Signals to Developmental Timing in *Drosophila* *Mikkal Blick*[1], Yuya Ohhara[2], Riyan Bittar[3], Michael O'Connor[4], Naoki Yamanaka[3]. 1) Genetics, Genomics, and Bioinformatics Program, University of California, Riverside, Riverside, CA; 2) School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan; 3) Department of Entomology, University of California,

Riverside, Riverside, CA; 4) Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN.
Drosophila melanogaster has multiple life stages, with the release of the steroid hormone ecdysone being responsible for developmental transitions from one life stage to the next. There are multiple controls for ecdysone release, including signaling by prothoracicotropic hormone (PTTH). PTTH, released by two pairs of bilateral neurons in the protocerebrum, plays a role in the timing of ecdysone release from the prothoracic gland, with a delay in development from third instar to pupariation caused by the ablation of the PTTH secreting neurons. Determining what factors signal for PTTH secretion would show what plays a role in the timing of development of *Drosophila* larvae to pupariation. Here, we show that the neurons producing the neuropeptide *hugin* in the subesophageal zone (SEZ) send signals to the PTTH neurons and contribute to the control of developmental timing in *Drosophila*. GFP Reconstitution across Synaptic Partners (GRASP) analysis suggests that the hugin neurons likely form synapses with the PTTH neurons. Additionally, null mutations of both *hugin* and the *hugin* receptor-encoding genes cause a delay in developmental timing similar to that caused by PTTH neuron ablation, and the receptor mutation phenotype can be rescued through expression of the *hugin* receptor driven by PTTH promoters. The SEZ is known to be innervated by gustatory receptor neurons (GRNs), which sense taste signals and have been shown to be involved in food preference. GRN signaling of the hugin neurons would potentially link external stimuli to developmental timing. Using gustatory receptor Gal4 lines, we have been able to show that multiple GRNs have a likely synaptic connection with the hugin neurons through GRASP analysis. Additionally, both the silencing and activation of some GRNs can alter the timing to pupariation. Taken together, our results suggest that PTTH control of developmental timing can be influenced by taste signals from the environment through a neuronal circuit that goes from GRNs to PTTH signaling via hugin neurons.

322A GWAS as a tool for identifying exercise response genes in *Drosophila Melanogaster* *Louis Watanabe*, Nicole Riddle. Biology, University of Alabama at Birmingham, Birmingham, AL.
Recent estimates show that more than 78.6 million adults are obese in the United States with the annual medical cost associated with this condition at $147 billion dollars in 2008. The most common treatments for obesity are dietary change or increased physical activity (exercise), or both. Despite the popularity of exercise as a treatment for obesity, very little is known about how an individual's genetic background impacts the response to exercise. In order to elucidate the genetic mechanisms underlying exercise response, we have developed an exercise system for *Drosophila melanogaster* named the Rotating Exercise Quantification System (REQS). This system allows for the real-time quantification of exercise in Drosophila. We use this system to measure activity levels in the Drosophila Genetics Reference Panel 2 (DGRP2). There is significant variability in exercise levels between these lines, with activity levels differing by at least one order of magnitude between the lines. Large differences were recorded also between males and females. Currently, data collection is more than 75% complete, and we will report the results of the on-going genome-wide association study. The results will reveal candidate genes that control exercise-induced activity levels and provide insights into the genetic architecture controlling this trait. This study validates the use of rotation-based exercise systems for Drosophila and demonstrates the potential of Drosophila as a model in the field of exercise genetics.

323B Host metabolism in determining microbiome composition using *Drosophila* mitonuclear introgression lines *Bianca R Brown*, David Rand. Ecology and Evolutionary Biology, Brown University, Providence, RI, 02906.
The microbiome of hosts in their natural habitat is species specific. This suggests that the host plays an active role in harboring its unique microbiome. The distinct microbiota provides a range of functions to its host. One in particular is regulating metabolism, the sum of energy producing chemical processes in a host, which maintains energy balance. In spite of the delicate balance that has to be maintained between the microbiome and its host, both the metabolism, and the microbiome, of a host organism are not static. Environmental factors influence homeostatic changes in metabolism, and these metabolic changes influence immune functions of the host, which can in turn alter microbiome abundance and composition.
This study asked how genetic manipulations of central metabolism can alter the host-microbiome symbiosis. In order to pursue this question a mitonuclear Drosophila introgression system, in which the mitochondrial and nuclear genomes are decoupled through genetic exchange crosses was used to study host metabolism through perturbation of the cross talk between the coevolved mitochondrial and nuclear genomes. A total of 6 mitonuclear genotypes were grown under sterile and laboratory conditions using respirometry. Differences in metabolic rates among sterile genotypes were observed. Sterile genotypes were then allowed to grow under normal laboratory conditions for five generations. 16srRNA sequencing of both males and females was done to assess microbiome composition of each generation. Genotypes were similar among abundant taxa, however rare taxa were disparate among genotypes.

324C Characterization of female reproductive tract secretions in *Drosophila melanogaster* *Caitlin McDonough*, Scott Pitnick, Steve Dorus. Center for Reproductive Evolution, Biology Department, Syracuse University, Syracuse, NY.

In internally fertilizing species, the female reproductive microenvironment interacts with the male ejaculate in diverse ways that mediate reproductive outcomes. Secretions within the female reproductive tract (FRT) of *Insecta* have been associated with a variety of functions including lubrication, seminal fluid protein processing, sperm movement and storage, ovulation, re-mating behavior, immune response, gamete modification, and the production of a protective egg coating. Despite the ubiquity of FRT secretions little is known about their identity, source, or evolution. We used proteomics of FRT fluid and transcriptomics of the parovaria, a secretory gland, in addition to available expression data from reproductive and somatic tissues to characterize FRT secretions in *Drosophila melanogaster*. Enriched gene ontology categories of identified secretions include proteases, protease inhibitors and anti-microbial agents, thereby supporting established functions of female secretions in seminal fluid processing and postmating immune response. Many of these secretions have similar postmating expression patterns in multiple female reproductive structures, indicating a suite of redundant functions spatially distributed throughout the female reproductive tract. We also identified secretions with expression patterns distinct to the parovaria, suggesting unique functions of these glands. Secretions with digestive or odorant binding functions were often also expressed in other tissues such as the gut, salivary gland, or male accessory gland, which could be evidence of gene co-option or sub-functionalization. Function of these secretions in postmating changes in female physiology and fertility are currently being assessed using an RNAi knockdown approach.

325A Molecular Analysis of the Role of SOLO in Meiotic Sister Chromatid Cohesion Thomas Dockendorff, *Elsie Adams*, Bruce McKee. University of Tennessee, Knoxville. Biochemistry, Molecular and Cellular Biology Department. M407 Walters Life Sciences 1414 Cumberland Avenue Knoxville, TN 37996-0840.

Sister chromatid cohesion is necessary for proper segregation of chromosomes throughout meiosis. In yeast and other eukaryotes, sister cohesion is mediated by meiotic-specific cohesin complexes consisting of SMC1, SMC3, the a-klesin Rec8 and a member of the SCC3/SA family. The core elements of the cohesion ring complex SMC1 and SMC3 are essential for mitotic cohesion, but understanding of their role in meiosis is still limited in Drosophila. Moreover, no homolog of the a-kleisin Rec8 has been identified in Drosophila, and identity of any non-SMC subunits of meiotic cohesin complexes remains obscure. Nonetheless, the cohesion-cleaving enzyme Separase is required for chromosome segregation in Drosophila meiosis. Three Drosophila-specific and meiotic-specific proteins Sisters On The Loose (SOLO), Orientation Disruptor (ORD), and Sisters Unbound (SUNN), referred to as SOS proteins, are key components of the meiotic cohesion apparatus. *solo, ord* and *sunn* mutants show premature sister chromatid separation and high frequencies of chromosome nondisjunction at both meiotic divisions in female and male meiosis. SOS proteins localize to centromeres from the onset of meiosis and are required for centromere localization of SMC1 and SMC3. In females, these proteins are also required for regular levels of recombination; they localize to synaptonemal complexes (SCs) and are required for SC stability. The C-terminus of SOLO exhibits weak sequence similarity with a-kleisins, suggesting that it may be a functional homolog of Rec8. Consistent with this idea, SOLO has been shown to co-immunoprecipitate with SMC1 and interacts with both SMC1 and SMC3 by yeast two-hybrid analysis. To further address a possible role of SOLO as a meiosis-specific a-kleisin, we have extended the yeast two hybrid analysis. Yeast two hybrid assays showed that SOLO with deleted C-terminus and SOLO with deleted N-terminus interacts with SMC3 and SMC1. We further found that the C-terminus of SOLO interacts with SMC1 and its N-terminus interacts with SMC3. Also, full length SOLO, N-terminal SOLO, and C-terminal SOLO interact with SUNN, consistent with prior evidence that SOLO and SUNN are mutually dependent for centromere localization. In addition, we are using site-directed mutagenesis and transgene analysis to test the in vivo functionality of five potential Separase-cleavage sites in SOLO. If SOLO is the Rec8 homolog, then rendering it uncleavable by Separase should disrupt sister chromatid segregation. Taken together, our studies provide insight into the composition of the meiotic cohesion apparatus in Drosophila.

326B Role of *drop dead* in Spermatogenesis *Anika Benske*, Edward M Blumenthal. Biological Sciences, Marquette University, Milwaukee, WI.

Following terminal differentiation, spermatids undergo individualization, a process in which an actin-based individualization complex (IC) removes excess cytoplasmic content and encloses each spermatid in its own plasma membrane. While this process is important for fertility, it is not well understood. It has been shown that individualization is sensitive to temperature, age, and changes in fatty acid derived signals (Ben-David et al., 2015). The *drop dead* (*drd*) gene encodes a proposed acyltranferase and is expressed in epithelial cells. Severe mutations in this gene lead to early adult lethality, gut dysfunction, and female sterility. No effects on male fertility have previously been reported for *drd* mutants. A deficiency screen for enhancers of the mild allele drd^{G3} identified a deficiency, *Df(2L)drm-P1*, that not only enhances adult lethality but also causes synthetic male sterility. To investigate the cause of this sterility, and to determine whether *drd* mutants also show similar defects, total and normal ICs were

quantified after staining testes of various *drd* mutants with fluorophore-conjugated phalloidin. Both drd^{G3} and drd^{G3}; *Df(2L)drm-P1*/+ males show reduced total ICs and an increased proportion of abnormal ICs compared to wildtype (WT) Canton S. Despite a greater proportion of abnormal ICs, mature sperm are still visible within the seminal vesicle of sterile drd^{G3}; *Df(2L)drm-P1*/+ mutants. Global knockdown of *drd* by RNAi, which does not cause male sterility, results in no change in total ICs but an increase in the proportion of abnormal ICs compared to sibling controls. These data demonstrate a previously unknown role of *drd* in male fertility and suggest that the gene functions both during spermatid individualization and at some point downstream of this process. Supported by NSF grant IOS-1355087.

327C Characterizing the role of *Ecdysone induced protein 74EF* in the length of sperm in males and seminal receptacle in females of *D. melanogaster* *Sharif O Chebbo*, Manier Mollie. Department of Biological Science, The George Washington University, Washington, DC.

Sperm of *D. melanogaster* are among the longest known and are evolving in concert with the female's long, coiled sperm storage organ, the seminal receptacle (SR). During sperm competition, long sperm outcompete short sperm but primarily in long SRs, providing a post-copulatory analog to male trait-female preference coevolution, commonly modeled in pre-copulatory sexual selection. Sperm and SRs are also likely coevolving via Fisherian runaway sexual selection, as evidenced by a recently discovered genetic correlation between these two traits. We previously identified a number a number of candidate genes influencing sperm length using a RAD QTL sequencing approach and have prioritized *Ecdysone induced protein 74EF (Eip74EF)* due to its role in autophagy during development, post-meiotic expression during spermatogenesis (when spermatid elongation occurs), the presence of multiple SNP markers within the gene, and signatures of rapid evolution under positive selection among the 12 *Drosophila* genomes. We have found that knockout mutant males have shorter sperm, suggesting that *Eip74EF* acts on the positive regulation of sperm length. We are also investigating female mutants to determine if a similar pattern exists for SR length. If so, *Eip74EF* may have pleiotropic effects on both sperm length and SR length and may therefore be a key to the molecular mechanism of Fisherian runaway sexual selection. Both male and female mutants also have decreased fertility relative to control flies, and males have reduced sperm competitive success, consistent with the expectation that shorter sperm are weaker competitors.

328A Two novel related genes required for post-meiotic mitochondrial shaping in *Drosophila* spermatogenesis *Katherine Copenhaver*, Karen G Hales. Department of Biology, Davidson College, Davidson, NC.

Homozygous males from the *Z2-2588* strain of *Drosophila melanogaster*, from the Zuker collection, are sterile, with abnormal nebenkern morphology and defective mitochondrial elongation during spermatogenesis. We identified the gene that contains this novel, uncharacterized mutation, and further characterized the mutant phenotype. We localized the *Z2-2588* mutation on the second chromosome within overlapping deficiencies that failed to complement the mutation, and we identified thirteen candidate genes within this region. The highly expressed, testis-specific genes *CG5043* and *CG5050* were sequenced in *Z2-2588* homozygous mutant males and compared to the DNA sequence for these genes in a different strain with the same background chromosome. The single base pair difference was a nonsense mutation in the mutant strain which is predicted to truncate the *CG5043* gene product. To further confirm this candidate gene, we conducted RNAi experiments, using two different RNAi constructs for both *CG5043* and *CG5050*. Only the male offspring from one cross containing the bam-GAL4 driver and the *CG5043* RNAi construct were sterile and showed similar mitochondrial defects to homozygous *Z2-2588* males, supporting *CG5043* as the gene associated with the phenotype. Knockdown of *CG5050* showed separate mild nebenkern abnormalities. The *CG5043* and *CG5050* gene products are similar to each other but novel with no similarities to any characterized proteins.

329B Mitochondrial-Nuclear Interactions and the Thermal Sensitivity of Male Reproduction *Abhilesh Dhawanjewar*, Colin Meiklejohn, Kristi Montooth. University of Nebraska-Lincoln, Lincoln, NE.

Temperature is a critical ecological parameter that influences physiological processes involved in growth, development and reproduction and, in turn, determines the distribution and abundance of most animal species. Spermatogenesis appears to be sensitive to temperature in all animals and this thermal sensitivity may have particularly important implications for the adaptation of ectothermic species to their local environments. Male sterility temperature thresholds show considerable variation within and between *Drosophila* species that correlates with geographical distributions and local climatic conditions. Male *Drosophila* typically show sharp critical thermal limits to fertility and this temperature-induced sterility is reversible, as males recover fertility after a few days at permissive temperatures. We are investigating the role of mitochondrial-nuclear interactions in shaping thermal-sterility thresholds, using strains with controlled nuclear genotypes and that carry mtDNAs from *D. melanogaster* or *D. simulans*. Our preliminary results indicate a role for developmental timing in thermal sterility. All genotypes were maintained at a permissive temperature of 25°C; flies were then transferred as eggs, third-instar larvae or adults to

29°C and, upon eclosion, males of each genotype were housed with 3 virgin females from a control genotype known to be fertile at 29°C. All genotypes were fertile when the males were transferred as adults and all genotypes were sterile when the males were transferred as eggs. This pattern suggests a role for early germline development, rather than adult spermatogenesis, in causing thermal sterility. When the males were transferred as third-instar larvae, we observed differential sterility across mitochondrial-nuclear genotypes. These preliminary data suggest that thermal sterility is sensitive to the life stage at which the flies experience thermal stress and also implicate mitochondrial-nuclear interactions in shaping these thermal-sterility thresholds.

330C Dynamic expression of an architectural protein Suppressor of Hairy-wing is required in spermatogenesis *Tingting Duan*, Pamela Geyer. Biochemistry, University of Iowa, Iowa City, IA.

Spermatogenesis is a complex process that produces mature sperm following an ordered differentiation process. In Drosophila, the differentiation process is accompanied with changes in nuclear size and architecture that facilitate transcriptional regulation and meiotic chromosome segregation. We have shown that the multifunctional transcription factor, Suppressor of Hairy-wing [Su(Hw)], is required for male fertility. Immunohistochemical analyses revealed that Su(Hw) is dynamically expressed during spermatogenesis, present in early stages and down-regulated just prior to the onset of the testis-specific differentiation program. Based on this observation, we tested the prediction that Su(Hw) contributes to transcriptional regulation of spermatogenesis. We identified ~300 genes mis-regulated in *su(Hw)* mutants. Most of the mis-regulated genes are de-repressed somatic genes, with few testis-specific transcripts are disrupted. In addition to being a transcriptional regulator, Su(Hw) is a nuclear architectural protein required for homolog pairing in somatic tissues. For this reason, we have begun to test the prediction that Su(Hw) plays a role in establishing homologue associations and chromosome territories during male meiosis. Our emerging data indicate that in *su(Hw)* mutants homologues display more unpairing at pericentric heterochromatin regions. Future work will aim to define the extent and consequence of this disruption. Together, our studies suggest that Su(Hw) makes multiple contributions during spermatogenesis, which are needed for male fertility.

331A Characterization and rescue of *mulet*, a mutation that disrupts spermatid individualization in *Drosophila melanogaster* *James Fabrizio*[1], Ferrara Elisa[1], Mendoza Gabriela[1], Rodway Stephanie[1], Vicioso Matthew[1], Villanobos Marielle[1], Rollins Janet[1], Buchner Erich[2], Wegener Stephanie[2], Bazinet Christopher[3]. 1) Division of Natural Sciences, College of Mt St Vincent, Bronx, NY; 2) Institut für Klinische Neurobiologie Universitätsklinikum Würzburg Versbacher Str. 5 97078 Würzburg; 3) Dept of Biological Sciences St. John's University, NYC.

Proper spermatid individualization requires the coordinated movement of 64 actin-based investment cones along the spermatid flagella. This coordination is severely disrupted in *mulet* mutant testes, resulting in a failure of spermatid individualization and male sterility. Recently, *mulet* was found to encode Tubulin-binding cofactor E-like (TBCEL), a chaperone responsible for the disassembly of microtubules. Moreover, fluorescence microscopy revealed the persistence of a population of inter-flagellar microtubules in *mulet* mutant testes suggesting that this tubulin cofactor may be responsible for removing these inter-flagellar microtubules as a prerequisite for proper spermatid individualization. In order to further characterize the *mulet* mutant phenotype, *don juan-GFP (dj-GFP),* a known molecular marker of spermatid mitochondria, was crossed into the *mulet* mutant background. While in wild-type testes, whorls of mitochondria visualized by *dj-GFP* were always associated with the F-actin based investment cones of the individualization complex, there was a lack of consistent association between the two in mutant testes. In addition, electron microscopy also revealed the presence of inter-flagellar microtubules in *mulet* mutant testes, consistent with our previous findings and strongly suggesting that individualization fails as a result of the persistence of these microtubules. Finally, a restoration of the wild-type phenotype was accomplished using *tubulin-Gal4* to drive expression of TBCEL in the testes, indicating that the mutant phenotype is indeed caused by the lack of TBCEL and TBCEL is specifically required for spermatid individualization. Taken together, these results suggest that TBCEL is required for both proper association between the individualization machinery and the spermatid mitochondria and the removal of inter-flagellar microtubules during spermatid individualization in Drosophila.

332B Characterization of a *Drosophila* ortholog of *SLC25A46* which is required for mitochondrial shaping during spermatogenesis *Vivienne Fang*, Karen Hales. Department of Biology, Davidson College, Davidson, NC.

Mitochondria undergo various shape changes during *Drosophila* spermatogenesis, making this process useful for studying mitochondrial morphology. Aberrant mitochondrial clumping in late-stage spermatid elongation occurs in *CG5755*$^{-/-}$ mutants. A recessive male sterile nonsense mutation in *CG5755* was discovered after deficiency mapping of the *Z2-3738* strain from the Zuker collection and subsequent identification and sequencing of the only testis-specific gene within the candidate region. The mutation was absent from *CG5755* in a different strain with the same background chromosome. Crosses to trigger RNAi knockdown of *CG5755* in testes produced males with wild type mitochondrial shaping, but since insufficient knockdown was a possibility, subsequent experiments are incorporating UAS-Dicer for an enhanced effect. To investigate the possibility of partial redundancy, we tested

genetic interactions between *CG5755* and its broadly expressed paralog, *CG8931*. Male *CG5755* heterozygotes that are hemizygous for a non-lethal insertion in *CG8931* (X linked) are fertile, though the insertion may enhance the *CG5755* homozygous phenotype. *CG5755* encodes a predicted member of the mitochondrial solute carrier family, though as predicted by homology, its function may have diverged. The human ortholog, *SLC25A46*, was found by other research groups to localize to the mitochondrial outer membrane, to interact with mitofilin, and to be associated with fusion/fission dynamics; *SLC25A46* is associated with optic atrophy spectrum disorder.

333C Fertility and sperm storage in aged *Drosophila* males *Solomon Friedman*, Josefa Steinhauer. Department of Biology, Yeshiva University, New York, NY.

Age related declines in fertility are observed in humans and other animals. Age related declines in female fertility have been well studied, but less is known about male fertility decline. In *Drosophila* males, germline stem cell number and proliferative capacity decline with age. Consistent with this, our lab has observed that germline cyst production declines steadily throughout the life of the fly. Surprisingly, our initial measurements of male fertility did not show a coincident drop with age, even at 30 days. This was determined to be due to storage of sperm throughout the lifetime of the males. When males were mated throughout the course of their life to deplete sperm storage, fertility was seen to decline at 30 days, but not at 15 days. Seminal vesicles of unmated males were significantly larger than those of mated males, confirming that sperm were stored. In males aged for 15 days, average cyst number per testis was significantly reduced compared to younger flies but was similar between mated and unmated males, despite large differences in seminal vesicle size. This suggests that seminal vesicle size and sperm storage do not feed back on cyst production. We conclude that sperm production in *Drosophila* males declines steadily and consistently with age, which likely influences male fertility in the wild. We are interested in the regulation of sperm storage and potential age related declines in sperm viability.

334A Characterizing the Role of Rough Deal (Rod) Protein in *Drosophila* Male Meiosis *Qiutao He*, Bruce D. McKee. Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN.

Meiosis is a specialized cell division in sexual reproduction which produces the haploid gametes from diploid precursor cells. Two separated stages are involved in this process – meiosis I and meiosis II. Accurate homolog segregation in meiosis I and sister-chromatid separation in meiosis II enable the genetic fidelity and prevents the generation of aneuploidies. Spindle assembly checkpoint (SAC) is a surveillance mechanism to ensure completion of bipolar alignment before chromosome segregation and the role of SAC in meiosis has not been fully characterized. Rough Deal is a protein involved in SAC among higher eukaryotes and contributes to the recruitment and removal of other SAC-related proteins on kinetochores during cell division. In *Drosophila* mitosis, mutations in *rod* lead to stretched chromatid arms, anaphase lagging chromatids and chromosome mis-segregation while null mutations are lethal. In present study, we took advantage of a fully viable meiosis-specific *rod* allele to assess the role of *rod* in *Drosophila* male meiosis. Sex chromosome nondisjunction (NDJ) analysis revealed that the majority of NDJ event occurred between homologous chromosomes in meiosis I (95%) compared to that between sister chromatids in meiosis II (5%). Interestingly, our FISH data clearly showed that all the sister chromatids of sex chromosomes held together normally until metaphase I while perturbed chromatid distribution was observed in 30% of spermatocytes during anaphase I among which equation segregation rate was as high as 5%. In addition, cytological data exhibited more than eight centromeres in spermatocytes from metaphase I to anaphase I and the maximum number of centromeres was twelve suggesting that autosomal sister chromatids separated prematurely in meiosis I. Consequent FISH result revealed that sister chromatids of 3rd chromosome separated precociously in 8% of metaphase I spermatocytes and this rate surged to 43% in anaphase I. Besides disrupted chromosome disjunction, lagging chromosome can also be observed in 21% of spermatocytes in anaphase I. Future studies will be split into two directions. Further effort will focus on how mutations in *rod* affect centromeric cohesion on autosomes and whether a disrupted sister-chromatid mono-orientation could result in the equational segregation of sex chromosome in *rod* mutants.

335B Investigating spermatogenesis in the haplodiploid jewel wasp Nasonia vitripennis *A. Jing*, P. M. Ferree. W. M. Keck Science Department, Claremont McKenna, Scripps and Pitzer Colleges, Claremont, CA.

Although much research effort has focused on understanding gametogenesis in Drosophila melanogaster and other traditional models, little is known about variations of gametogenesis outside these groups. The jewel wasp, Nasonia vitripennis, is a rising model organism representative of the prominent insect order Hymenoptera, which includes all wasps, bees, ants, and sawflies. This group exhibits a form of reproduction known as haplodiploidy, in which females arise as diploids from fertilized eggs whereas males arise as haploids from unfertilized eggs. We performed a detailed analysis of several specific processes involved in spermatogenesis in N. vitripennis by using different microscopic approaches. The sperm bundles contain widely varying sperm cell numbers, in dramatic contrast to D. melanogaster, whose bundles invariantly contain 64 sperm. We propose that this variance reflects mitotic divisions that can safely produce haploid sperm cells through mitosis only, no matter how many divisions occur per cyst. In

support of this idea, we found no evidence for somatic cyst cells, which in D. melanogaster tightly regulate ordered cyst divisions. Instead, we detected highly polyploidy cells, similar to nurse cells in Drosophila egg chambers, which are interspersed among the dividing cysts of germ cells. These cells may signal germ cell division at a distance from within the wasp testis. Finally, we found that the acrosome vesicle does not cross-react with lectin reagents, suggesting that it does not contain N-acetylglucosaminyl sugar residues, unlike D. melanogaster. These findings begin to underscore substantial variations in gametogenesis between dipterans and hymenopterans.

336C Evaluating ring canal function during spermatogenesis *Ronit Kaufman*, Kathleen Ayers, Andrew Hudson, Allison James, Lynn Cooley. Yale University, New Haven, CT.

Intercellular bridges, or ring canals (RCs), connect germline cells during gametogenesis in males and females. RCs form as a consequence of incomplete cytokinesis at the end of mitosis leaving daughter cells connected by a stable intercellular bridge of cytoplasm. The function of RCs is best understood in Drosophila females where they allow movement of cytoplasm from the nurse cells into growing oocytes. Despite their widespread presence in animal testes from insects to mammals, surprisingly little is known about the role of RCs and intercellular movement of cytoplasm during spermatogenesis. In Drosophila males, a single germ cell divides mitotically four times to form a 16-cell cluster of spermatogonia. The cells complete S-phase of meiosis I to become spermatocytes and eventually undergo meiosis II to form a 64-cell cluster of haploid spermatids still connected by RCs. Several hypotheses for this perdurance of RCs have been proposed including enabling the movement of X-linked gene products into post-meiotic Y-bearing spermatids, coordinating mitotic and meiotic cell cycles, or sharing signals about the overall health of the developing syncytium. We have developed live imaging approaches to study intercellular movement through RCs to determine if male RCs allow for movement of proteins between connected cells. We have demonstrated movement of photoactivatable GFP (Pa-GFP) in cysts from the 2-cell spermatogonia all the way through fully formed spermatids. Moreover, we have used Fluorescence Loss in Photobleaching (FLIP) to show diffusion of cytoplasmic GFP and endogenous GFP-tagged proteins between connected cells in a cyst. To address coordination of cell behavior, we are using live-cell imaging to characterize mitotic cell cycle transitions within interconnected cysts using the Fly-FUCCI system. Our results reveal extensive sharing of cytoplasmic information that likely supports coordinated behaviors.

337A Testis-specific ATP synthase subunits associated with shaping mitochondrial membranes in the nebenkern *Jonathan Lim*, Elizabeth Brunner, Eric M Sawyer, Lindsay Regruto, Karen G Hales. Department of Biology, Davidson College, Davidson, NC.

In post-meiotic *Drosophila* spermatids, mitochondria fuse into a spherical nebenkern with the membranes of the two topologically distinct compartments forming concentric spherical layers. We have identified *knon* as a gene required for nebenkern morphogenesis and sperm motility. *knon* encodes a testis-specific large paralog of the D subunit of F_1F_0 ATP synthase, a complex which shapes cristae by forming higher-order dimers in the inner mitochondrial membrane. Knon presumably can replace subunit d on the peripheral stalk of the F_1F_0 complex, where it could sterically hinder F_1F_0 dimerization, enabling ATP synthase-lined membranes to curve more freely in the nebenkern. Consistent with this hypothesis, expressing Knon exogenously in flight muscle causes a reduction of ATP synthase complex dimerization, as shown by BN-PAGE and immunoblotting. The *Drosophila* genome contains additional genes predicted to encode testis-specific paralogs of ATP synthase subunits, including subunits B, F6, and G. Since these three subunits also reside on the peripheral edge of F_1F_0, we predict that the testis versions may play a role similar to that of Knon in shaping the nebenkern by altering F_1F_0 dimerization. Indeed, RNAi knockdown of two of these genes causes alterations of mitochondrial morphology. Our ongoing mutagenesis of these genes will provide further information on the extent of the involvement of the testis-specific B, F6, and G subunits in shaping the nebenkern via mediation of ATP synthase dimerization.

338B Defective *Drosophila* spermatogenesis in *CG4701* and *nmd* mutants possibly connected to faulty protein transport and peroxisome biogenesis *Papa Kwadwo Morgan-Asiedu*, M. Ummer Qureshi, Karen G Hales. Department of Biology, Davidson College, Davidson, NC.

Mitochondrial shaping is a phenomenon that occurs in many organisms and can be studied extensively during *Drosophila melanogaster* spermatogenesis. The male sterile strains *nmd*$^{P[ry4]}$, *nmd*^{N162I}, and *CG4701* exhibit aberrant mitochondrial phenotypes. *nmd*$^{P[ry4]}$ mutants display aggregation failure, *nmd*^{N162I} and *CG4701* mutants have multiple nuclei per nebenkern during onion stage indicating failed meiotic cytokinesis, and *CG4701* mutants show vacuolated nebenkerns. Msp1, the *Saccharomyces cerevisiae* ortholog of CG4701 and Nmd, participates in mitochondria to peroxisome trafficking of Peroxin proteins (PEX), and we suspect that CG4701 and Nmd may have related roles. Pex proteins regulate biogenesis of peroxisomes which metabolize very-long-chain-fatty-acids (VLCFA). We visualized peroxisomal organization at each stage of spermatogenesis in wild-type and *CG4701*$^{-/-}$ flies. We observed that peroxisomes normally demonstrate stage-specific patterns of organization, including being positioned near mitochondria and microtubule organizing centers, sites associated with Nmd localization. *CG4701*$^{-/-}$

males sometimes demonstrated reduced stage-specific peroxisomal clustering, and we found fewer peroxisomes at the basal body from onion to elongation stage. In line with our hypothesis that defective peroxisome biogenesis is associated with defects in the *nmd* and *CG4701* mutants, *pex2 and pex13* mutants showed cytokinesis failure similar to that of *nmd* and *CG4701* mutants. To test for a peroxisomal function of *nmd* and *CG4701*, we developed protocols for overloading peroxisomes via VLCFA food supplementation; preliminary results show shriveled testes and significant defects in the onion stage of spermatogenesis in *pex13* mutants as compared to the LCFA controls, confirming the validity of the protocol, and allowing similar tests on *nmd* and *CG4701*. Finally, to test whether *pex* mutations compromise *nmd* and *CG4701* functions, we are comparing localization of Nmd-GFP and CG4701-RFP in *pex* mutants to their localization in WT. Our results thus far support the mitochondria-peroxisome protein transport hypothesis of Nmd and CG4701 function.

339C Characterizing the effects of *kayak* knockdown on post-copulatory sexual selection in *D. melanogaster* *Jai P. Photavath*, Mollie K. Manier. The George Washington University, Washington, DC.

In *Drosophila,* the lengths of male sperm and female sperm storage organs (seminal receptacle; SR) play major roles in post-copulatory sexual selection (sperm competition and cryptic female choice), are coevolving, and may be involved in rapid evolutionary diversification within and among species. Despite their significance, the underlying genetic mechanisms for sperm and SR length variation are not well understood. Recent work has uncovered a genetic correlation between the two traits, suggesting that they are coevolving under Fisherian runaway sexual selection, as is predicted for pre-copulatory sexual selection on male traits and female preferences for those traits. Previous work identified a number of candidate genes for sperm length using a RAD QTL approach, and we have characterized the phenotypic effects of a *kayak* knockdown in *D. melanogaster* using RNAi. *kayak* is involved in transcriptional regulation and signal transduction during embryonic development; however, it has no known roles in spermatogenesis. Knockdown of *kayak* increased both sperm and SR length, suggesting that the gene acts as a negative regulator for these phenotypes. Although sperm head and tail lengths are not known to be pleiotropic, the sperm head lengths of knockdown males were also significantly longer than those of control males. A decrease in fertility rates was observed, supporting previous evidence of longer sperm delaying reproductive maturity and costing more energy to produce. *kayak* also demonstrates strong allelic divergence between isolines with long and short sperm. The roles of *kayak* in mating and re-mating behavior, sperm competition, and sperm storage ability are currently being investigated through RNAi knockdown. This study is important for understanding the underlying genetic mechanisms of spermatogenesis and sperm storage organ development as well as characterizing molecular mechanisms of Fisherian runaway selection and male-female coevolution.

340A Small Ubiquitin-like Modifier (SUMO) Posttranslational Modifications Mediate Critical Regulatory Events during *Drosophila* Spermiogenesis and Spermatogenesis *Janet Rollins*[1], Kemsil Hwang[2], Patricia Morris[2,3]. 1) Natural Sci, Col Mt Saint Vincent, Riverdale, NY; 2) Biomedical Research, Population Council, New York, N.Y; 3) The Rockefeller University, New York, N.Y.

Drosophila spermatogenesis is a dramatic, temporally-orchestrated developmental stage-specific process. Sperm production includes marked changes in mitosis and meiosis, chromosomes, transcription, translation, and posttranslational modifications, with striking nuclear remodeling during spermiogenesis. The posttranslational small ubiquitin-like modifier (SUMO) protein has been shown to play diverse roles in many highly conserved cellular processes such as spermatogenesis in various species including man and rodents. The purpose of this study was to define the precise stage-specific timing of fly Smt3 (*Drosophila* SUMO)-mediated events during germ cell development, determine whether Smt3-deficiency affects sperm production, and initially identify Smt3-modified proteins for comparison with those during mammalian spermatogenesis. For bioimaging, unconjugated Smt3 and Smt3-modified proteins were detected by immunofluorescence using both whole mounts and squash preparations of testis from wild type and heterozygous Smt3-deficient mutant stocks, avoiding homozygous larval lethality. Smt3- SUMOylated proteins were determined by immunoprecipitation (IP) and Western blot analyses. Protein extracts prepared from human and rodent testes were used for comparison. Male fertility of fly mutants was assessed by matings with wild-type females. In wild-type flies, Smt3-SUMOylated proteins show strikingly different patterns in most stages of spermatogenesis including spermatogonia undergoing mitosis, resting and meiotically active spermatocytes, and round and elongating spermatids in various stages of nuclear condensation during spermiogenesis. Smt3 was not detected in hub cells. The testes of heterozygotes showed reduced levels of Smt3 and an altered SUMOylated protein profile compared to wild type. Interestingly, the reduction of Smt3 signals was readily observed in meiotic spermatocytes; no change for mitotic spermatogonia was apparent. Heterozygote males exhibited a reduced fertility and their testes show a marked defect in sperm transfer to the seminal vesicles. Smt3-modification of an essential cell cycle kinase was observed only during anaphase-to-telophase transition. Our data are suggestive that 1) precise timing of SUMOylation events in developing fly germ cells is required for normal spermatogenesis; 2) Smt3-deficiency can result in failure of spermatids to properly undergo spermiogenesis and sperm transfer, findings consistent with marked reduction in fertility. Taken together, our results indicate important roles for Smt3 and SUMOylation during and after meiosis in*Drosophila* testis.

341B Identification of Lipid-Processing Genes Required for Eggshell Formation in *Drosophila* *Kalyn Gackowski*, Edward M Blumenthal. Biological Sciences, Marquette University, Milwaukee, WI.

Lipid processing is known to be essential for female fertility in animals from insects to humans, yet information is lacking on many of the diverse ways through which lipids and lipid-derived signals are required for fertility. The main purpose of this study was to identify lipid-processing genes whose expression in the somatic follicle cells of the ovary is necessary for formation of the vitelline membrane (VM) of the eggshell. Potential targets for the screen were

identified from a microarray database of gene expression during late oogenesis (Tootle et al., PLoS One, 2011); targeted genes were those with significantly higher expression at stage 10B of oogenesis, the stage at which VM proteins are synthesized, compared to stage 12, and that encoded proteins with lipid-processing or unknown function. To date, a total of twenty nine genes have been screened by driving RNAi with the follicle cell-specific driver *CY2-gal4* and looking for female sterility. Two hits were identified. The first, *Fatp,* which encodes a fatty acyl-CoA synthase, resulted in a severe collapsed egg phenotype and total sterility when knocked down. Knockdown of the second, *CG5065*, which encodes a fatty acyl-CoA reductase, resulted in total sterility but had a less severe effect on the integrity of laid eggs. Ongoing experiments will look specifically at the role of these two genes in the expression and crosslinking of VM proteins. These data provide further evidence that lipid-processing genes, specifically *Fatp* and *CG5065,* play an essential role in eggshell formation. This project was supported by NSF grant, IOS-1355087.

342C Autocrine Jak/Stat signaling is required in the polar cells *Michelle Giedt*, Douglas Harrison. University of Kentucky, Lexington, KY.

The polar cells are required for multiple events during oogenesis including follicle cell specification and border cell migration through activation of Jak/Stat signaling. However, relatively little is known about their functions after completion of border cell migration. It has been observed that the polar cells form protrusions into the developing micropyle to form the micropyle lumen. However, the mechanisms underlying the formation and guidance of the polar cell extensions are unknown. We observed that *upd3* mutants have an increased incidence of unfertilized eggs and a small but significant fraction of eggs from mutant females have blocked micropyles. The polar cell/border cell cluster still migrates to the anterior of the oocyte, but the polar cell extension misses the micropyle resulting in blockage. Here we present evidence that autocrine Jak/Stat signaling is required for appropriate extension formation. Imaging of live and fixed ovaries, shows that the polar cell extension forms in a coordinated, predictable manner similar to axon outgrowth. Furthermore, the polar cell extension forms prior to appearance of the micropyle and seems to mark the location where the micropyle will form. While autocrine Jak/Stat activity in the polar cells is not required for process formation or migration of the border cell cluster, its reduction does affect extension morphology. Furthermore, regulators of cell polarity and cytoskeletal dynamics affect formation of extensions, similar to their functions in axon formation. Perturbation of these pathways is being used to impair formation of polar cell extensions to better understand the timing of their requirement and functions in micropyle formation.

343A Genetic Determinants of Germline Stability *Ethan Greenblatt*, Allan Spradling. Department of Embryology, Carnegie Institution of Washington, Baltimore, MD.

Quiescence represents a fundamental cellular state in which cells with reduced metabolic activity remain viable for long periods of time. Germ cells alternate between periods of proliferation and quiescence in many animals, and some germ cells - such as those found in primordial follicles in humans – spend the majority of their existence in a developmentally arrested, quiescent state for several decades prior to activation. How germ cells maintain their viability during quiescent periods and remain capable of responding rapidly to external cues is unclear.
We are using Drosophila as a model system to understand the genetic requirements of germline quiescence. Drosophila have been reported to retain mature oocytes under unfavorable conditions, and these retained oocytes are transcriptionally and translationally repressed. By controlling nutrient availability and access to males, we established an assay in which the length of retention of oocytes was precisely controlled over the course of several weeks. Using this system, we find that wild-type females can retain oocytes for ~12 days with a 50% loss of viability at 25C. Oocyte viability during retention is strongly temperature-dependent, as 50% loss of oocyte viability occurs after only ~6 days at 29C.
In order to identify genes essential for germline maintenance during oocyte retention, we took advantage of the Gal4-UAS system to drive RNAi specifically in the female germline. In a targeted genetic screen, we identified the Drosophila homolog (dFmr1) of human Fragile-X mental retardation protein – a gene linked to the disorder of aging known as Fragile-X associated primary ovarian insufficiency – as a factor which is essential for the maintenance of arrested oocytes. Surprisingly, germline dFmr1 is entirely dispensable for the ability of newly synthesized oocytes to undergo embryonic development following fertilization, but becomes essential upon retention, as its loss leads to severe neuronal defects in embryos derived from aged arrested oocytes. Ribosome profiling of wild-type and Fmr1-deficient oocytes demonstrated that Fmr1 functions as a translational activator, promoting the translation of a small subset of oocyte-expressed mRNAs. Loss of Fmr1-target genes results in premature oocyte decline. These data identify a requirement for the continued translation of a set of "pilot light" genes in the maintenance of quiescent germ cells.

344B Regulation of Dlp cleavage by *Drosophila* Mmp2 *Indrayani Waghmare*, Xiaoxi Wang, Bryan Cawthon, Andrea Page-McCaw. Department of Cell and Developmental Biology, Vanderbilt University, Nashville-TN.

The apical cells of the germarium in *Drosophila* ovary are the source of secreted ligands such as Wingless (Wg) and Hedgehog (Hh). In *Drosophila*, the spread of Wg from these anterior niche cells is required for follicle stem cell

proliferation. HSPGs (Heparan Sulphate Proteoglycans) are known to regulate the distribution of secreted ligands during development. Dally like protein (Dlp) is one such cell surface HSPG. Dlp promotes long-range Wg signaling by 'trapping' extracellular Wg and presenting it to its cognate receptor-Frizzled (Fz) on cells that are farther away from Wg producing cells, and our previous work demonstrates that Dlp promotes long-range Wg signaling to the follicle stem cells. Using genetic tools in *Drosophila*, our lab further identified that Mmp2 inhibits Dlp mediated long range Wg signaling for follicle stem cell proliferation. Mmp2 is one of the two matrix metalloproteases found in flies and is localized at the cell surface via its glycosylphosphatidylinositol (GPI) anchor. Further, in cell-culture experiments, Dlp is cleaved by Mmp2 in the N terminal region. MMPs act on a wide range of substrates. However, they do not have consensus cleavage sites. In this study, we aim to 1) identify Mmp2 cleavage site(s) on Dlp using a proteomic approach (Mass Spec) and 2) test if Dlp cleavage occurs *in vivo* in an Mmp2 dependent manner. The identification of Mmp2 cleavage site(s) on Dlp, and understanding how this cleavage restricts the Wg ligand gradient will provide mechanistic insights into the regulation of long-distance signaling.

345C Null mutants for calcium independent phospholipase A_2 show normal male fertility but reduced female fertility *Adina Wakschlag*[1], Sogol Eizadshenass[1], Mindong Ren[2], Michael Schlame[2], Josefa Steinhauer[1]. 1) Department of Biology, Yeshiva University, New York, NY; 2) Department of Cell Biology, NYU Langone Medical Center, New York, NY.

Phospholipids are integral structural components of cells, constituting the majority of both the plasma membrane and organelle membranes. However, they are not inert or static; membrane phospholipids are dynamically reorganized and biochemically remodeled in response to signaling pathways and changing cellular behaviors. They also can be cleaved to release potent lipid signaling molecules (mediators), which can initiate signal transduction cascades. One important phospholipid remodeling pathway is the Lands Cycle, in which phospholipases A_2 (PLA_2s) cleave phospholipids at the *sn-2* position, releasing lysophospholipids and free fatty acids, both precursors to lipid mediators. This pathway has been implicated in fertility, brain development, inflammation, metabolism, mitochondrial function, cell death, and cancer. PLA_2s are conserved in *Drosophila* but have not been well characterized. We have determined the expression pattern of the calcium-independent phospholipase A_2 ($iPLA_2$) gene *CG6718* and generated a null mutation. $iPLA_2$ is expressed ubiquitously at low levels in imaginal tissues, and null mutants are viable, consistent with mouse knockout models. Surprisingly, $iPLA_2$ null mutants show no significant molecular species changes in cardiolipin or other phospholipids, despite previous suggestions that $iPLA_2$is critical for cardiolipin remodeling and phospholipid "housekeeping." These results indicate that cardiolipin and glycerophospholipid remodeling do not strictly require $iPLA_2$, possibly owing to genetic redundancy amongst PLA_2s or alternative phospholipid metabolism pathways. Although *Drosophila* $iPLA_2$ is highly expressed in the male germline and $iPLA_2$ knockout mice are male sterile, our $iPLA_2$ mutants are fully male fertile. However, our $iPLA_2$ mutants show reduced female fertility, and $iPLA_2$ is highly expressed in the female germline. This is in accord with previously demonstrated roles for phospholipid-derived mediators in oogenesis and female fertility. $iPLA_2$s are major targets for pharmaceutical intervention in humans, and their investigation in *Drosophila* will shed light on their functions and mechanisms.

346A Defining the RNAs of Drosophila germ cell precursors throughout development *Paul M. Albosta*[1], Phillip D. Zamore[1,2]. 1) RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA; 2) Howard Hughes Medical Institute.

In the reproductive tissues of animals, small silencing RNA pathways regulate germline development and fertility. These pathways use small non-coding RNAs to guide Argonaute proteins to their RNA targets, which are silenced via multiple mechanisms. In *Drosophila melanogaster,* establishment and maintenance of pole cells, the initial germ cell precursors, requires Argonaute proteins from each of the three animal small RNA pathways: miRNA, siRNA, and piRNA. Despite our growing understanding of how small RNAs are made and how they direct Argonaute proteins to silence transposons and protein-coding genes—by repressing their transcription or destabilizing or blocking translation of their mRNAs—we know little about the identity or function of the targets of small RNAs in germline progenitor cells. Moreover, we lack a comprehensive catalog of the small RNAs, mRNAs, and noncoding RNAs present in germ cell precursors across the development of the fly. Such a catalog is a prerequisite for understanding how small RNA-guided silencing contributes to the establishment, maintenance, and differentiation of germ cell precursors.

Using genetics, cell sorting, and deep sequencing, we are working to define the small and long RNA repertoire of the germ cell precursors of *Drosophila* as they develop from embryonic pole cells to adult germline stem cells. We seek to identify the small RNAs, mRNAs, long non-coding RNAs, and small RNA-directed target cleavage products present in distinct germ cell precursor populations in embryos, larvae, and adults. For example, we find that pole cell sequencing reads are dominated by piRNAs rather than siRNAs or miRNAs. The piRNA/miRNA ratio for pole cells sorted from 1–2 h embryos is 48-fold higher compared to age-matched whole embryos and 16-fold higher compared to age-matched unfertilized eggs. This asymmetric localization of small RNAs to pole cells suggests an important role

for piRNAs during early pole cell establishment or differentiation. Our observations are consistent with previous research showing that maternal inheritance of piRNAs is required for adult fertility, suggesting that these small RNAs guide the proper programing or differentiation of embryonic germline stem cells.

347B Understanding the *cis*- and *trans*-regulation of *Sex lethal* in the *Drosophila melanogaster* germline *Raghav Goyal*, Ellen Baxter, Pradeep Kumar Bhaskar, Mark Van Doren. Department of Biology, 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 dictated by its sex chromosome constitution. Interestingly, when *Sxl* is expressed in XY (male) germ cells, they are able to produce eggs upon transplantation into an XX (female) somatic gonad, demonstrating that even in the germline, *Sxl* is sufficient to activate female identity [1]. In both the germline and soma the presence of two X chromosomes leads to *Sxl* expression and female fate. However, the mechanism of counting the X chromosomes in the soma and the germline appears to be different at both ***cis*** and ***trans*** levels, and we are studying how this is done in the germline. The DNA elements regulating *Sxl*'s sex-specific promoter (***SxlPe***) activity in the germline have not been identified. To identify these, we are cloning DNA fragments covering the entire region upstream of *SxlPe*'s transcriptional start site into an enhanced GFP reporter vector to test for sex-specific expression in the germline. Further, we are performing RNA-FISH against nascent transcripts to understand the dynamics of *Sxl's* transcription initiation during embryogenesis. We are also investigating the *trans*-acting factors that regulate germline *Sxl* expression. Based on previous studies, the X chromosome "counting genes" important for activating *Sxl* in the soma are not required in a dose-dependent manner in the germline. However, we found that knocking down one of these genes, ***sisterless A*** (***sisA***), in the female germline does result in an ovarian tumor and germline loss phenotype, similar to masculinization of the germline due to loss of *Sxl* function. Encouragingly, *Sxl* expression is lowered in *sisA* RNAi ovaries and the germline loss phenotype can be rescued by *Sxl* cDNA. Finally, we are searching for additional *trans*-regulators of *Sxl* through an RNAi screen of genes expressed sex-specifically in the undifferentiated germline.

In both flies and humans, the sex of the germline must be properly coordinated with the sex of the soma. Since a failure to match germline and somatic sex leads to defects in gametogenesis, our work in understanding how proper sex determination leads to gonad development into one of two sexually distinct phenotypes is important for our understanding of reproductive biology and human health. [1] Hashiyama, K., et al. Science 333, no. 6044 (2011): 885-88.

348C Chinmo prevents Tra^F production in adult male somatic gonadal cells to preserve sex identity and fertility. *Lydia Grmai*[1], Erika Bach[1,2]. 1) Dept. of Biochem & Molecular Pharm, NYU School of Medicine, New York, NY; 2) Kimmel Center for Stem Cell Biology, New York, NY.

Reproduction in sexually dimorphic animals requires the production of healthy gametes. In *Drosophila*, gametogenesis is executed by the germline and aided by somatic support cells; autonomous and cooperative sex-specific signals in the germline and soma must converge on the same sex identity to ensure fertility. Somatic sex identity in *Drosophila* is instructed by sex-specific isoforms of the transcription factor Doublesex (Dsx). The RNA-binding protein Transformer (Tra^F) is necessary for female *dsx* splicing (Dsx^F), while default splicing in the absence of Tra^F generates male-specific Dsx^M.

Sex identity is critical in certain cell types for proper organ function, particularly in stem cells of the *Drosophila* testis. Loss of the BTB-ZF protein Chinmo in somatic stem cells causes them to "feminize", resembling somatic cells in the ovary by their morphology, arrangement, and temporal gene expression as they differentiate. In adult males, this somatic sex transformation creates a germline-soma mismatch and early block in spermatogenesis that renders the fly sterile. Genome-wide expression profiling of purified wild type versus *chinmo*-mutant somatic cells in the testis revealed that Chinmo represses the canonical sex determination pathway to preserve male sex identity in adults. *chinmo*-deficient somatic cells express female tra^F and dsx^F transcripts and knockdown of *tra* restores male identity in the absence of *chinmo*. Importantly, the tra^F produced in *chinmo*-mutant somatic cells is alternatively spliced by a Sxl-independent mechanism.

A search for candidate genes capable of altering *tra* splicing revealed low transcript levels of the RNA-binding protein *virilizer (vir)* in the adult testis and much higher levels in the adult ovary. Strikingly, *vir* expression is significantly enriched in *chinmo*-deficient testes. Preliminary results show that *vir* knockdown in *chinmo*-deficient somatic stem cells blocks feminization, suggesting it is required downstream of *chinmo* to splice *tra* pre-mRNA. Our results illustrate that active maintenance of male somatic sex identity in the *Drosophila* testis involves a novel mechanism for negatively regulating tra^F at both transcriptional and post-transcriptional levels. In summary, we have identified an essential role for Chinmo as a safeguard to prevent canonical female splicing in stem cells of the adult testis, protecting the ability of somatic support cells to properly differentiate and ensure spermatogenesis throughout the life of the organism.

349A Sex-specific development of the germline stem cell niche is regulated by a novel *doublesex* - *fruitless* regulatory interaction *Hong Zhou*, Cale Whitworth, Caitlin Pozmanter, Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore, MD.

In *Drosophila melanogaster*, sexual differentiation of the male and female gonads is controlled by the key sex-specific transcription factor Doublesex (DSX). While homologs of DSX are known to control sexual development in virtually all animals, including humans, the mechanism and downstream targets for these proteins remain largely unknown. Our genomic and bioinformatic approaches to identify DSX targets revealed *fruitless (fru)* as a candidate target for regulation by DSX. Previously it has been shown that *fru* acts in the nervous system to control sex-specific behaviors, and that male-specific expression of FRU is regulated by alternative splicing. Surprisingly, we found that FRU is also expressed in a male-specific manner in the developing somatic gonad. Further, sex-specific expression of FRU in the gonad does not require sex-specific alternative splicing and, instead, *dsx* is necessary and sufficient to regulate FRU expression in the male gonad. We also found that regulation of FRU expression by *dsx*, independent of alternative splicing, is important for sex-specific expression of FRU in the nervous system. We are currently analyzing the *fru* locus to test whether DSX is a direct transcriptional regulator of *fru* expression and whether this regulation is important for normal *fru* function.

A key aspect of establishing gonad sexual dimorphism is the formation of sex-specific germline stem cell (GSC) niches. Important components of these niches, the terminal filaments and cap cells (TFs/CCs) in females and the hub in males, originate from a common pool of progenitor cells but diverge to form sex-specific niches. Previously we have shown that, in the absence of *dsx* function, hubs are less robustly specified and stochastically switch to form TFs/CCs. Interestingly, FRU expression in the hub correlates with the time when the male niche must resist switching to form TFs/CCs. Further, ectopic FRU expression is able to interfere with normal TFs/CCs formation, and reduction of *fru* function causes an increase in the probability that hubs will switch over to TFs/CCs fate. We conclude that male-specific FRU expression is essential for maintenance of the male germline stem cell niche. In summary, this work demonstrates that *fru* expression is not only regulated by sex-specific alternative splicing but that *fru* expression is also regulated downstream of *dsx.* Further, *fru* not only acts to control sex-specific behaviors in the nervous system, but also functions in the somatic gonad to control sexual dimorphism of the GSC niches.

350B The PAR Complex is Required in Somatic Cyst Cells for Germ Cell Survival *Susanna Brantley*, Margaret Fuller. Developmental Biology, Stanford University, Stanford, CA.

In the *Drosophila* testis, two stem cell populations are maintained at the apical tip: germline (GSCs) and somatic cyst stem cells (CySCs). During germ cell differentiation, GSCs divide and enter a transit-amplifying (TA) program followed by onset of meiosis and terminal differentiation. CySCs divide once to produce a post-mitotic cyst cell. Two cyst cells enclose a cyst of proliferating germ cells and stay associated with this cyst through meiosis and terminal differentiation. Recent work has shown that without the CySC lineage, GSCs fail to enter the TA program. Here, we show that cyst cells are also required at the switch from proliferation to differentiation in the germ cell lineage. Using immunofluorescence and protein trap lines, we observed that components of the PAR complex, aPKC and baz, are expressed in cyst cells and localize to early cyst cell membranes. At later stages, cysts cell encapsulating post-mitotic spermatocytes, have a localized band of baz protein at the cyst cell-cyst cell junction. This band is also the location of adherens junction and basolateral domain proteins, suggesting that the cyst cell-cyst cell junction is structured like a simple squamous epithelial cell interface. Using cyst cell specific RNAi, we found that all three components of the apical PAR complex (baz, Par-6, and aPKC) are required in cyst cells for spermatocyte survival. However, cyst cells are not lost following PAR complex loss of function, and continue to encapsulate the remaining early germ cells and maintain a permeability barrier. We are currently investigating the hypothesis that adherens junction stability and associated signaling may be altered following PAR complex loss of function.

351C Identification of genes involved in oocyte specification and differentiation in *Drosophila* *Julie Merkle*, Trudi Schupbach. Princeton University, Princeton, NJ.

A fundamental question in biology is how cell fates are specified and maintained. In particular, the intricate process by which functional gametes are formed from the germline stem cells has yet to be unraveled. In *Drosophila*, oogenesis begins by asymmetric division of the germline stem cells, and after four mitotic divisions, produces a 16-cell cyst. One of these cells is selected as the oocyte, the future egg, while the remaining 15 become supporting nurse cells. In a genetic screen of lethal mutations in *Drosophila* using mosaic techniques, we identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination. Strikingly, egg chambers in which the germline cells are mutant produce cysts with 16 nurse cells and no oocyte. Two genes identified in this screen are *asteroid* (*ast*) and *Sec24CD*. Although mutation of each gene results in loss of oocyte identity, the stages at which they exhibit defects are different. This suggests that *ast* and *ghost*/*Sec24CD* are important during different steps of the oocyte fate differentiation process. The protein encoded by *ast* is predicted to be involved in DNA repair. Indeed, we observe persistence of double-strand breaks in *ast* mutant clones, indicating that meiosis-induced DNA damage is not properly repaired when *ast* is disrupted. *Sec24CD* mutant clones complete oocyte selection, however

oocyte identity is not maintained and the selected oocyte reverts to a nurse cell fate. *Sec24CD*, which encodes a COPII secretory coat component, is required for the generation of secretory vesicles at the ER. The discovery and further investigation of *asteroid*, *Sec24CD* and other previously unreported genes involved in various steps along the path to oocyte differentiation shed much needed light on this critical cell fate decision.

352A The role of CTP synthase in regulation of cell migration *Li-Mei Pai*, Pei-Yu Wang. Dept Biochem & Molec Biol, Chang-Gung Univ, Tao-Yuan, Taiwan.

Cytidine triphosphate synthase (CTPsyn) provides the CTP to form CDP-diacylglycerol (CDP-DAG) in phosphoinositides (PtdInsPs) synthesis pathway. Phosphatidylinositol (PI) is a glycerophospholipid, and a major component of all cell membrane. It is composed of a myo-inositol head group and acyl chain tails. The specific phosphoinositide kinases can phosphorylate the inositol ring to create mono-, bi, and tri-phosphate derivatives (PtdInsPs) of PI, such as PI(3,4,5)P_3, PI(4,5)P_2, and PI4P. These PtdInsPs mediate many processes in the cell, including signal transduction, cytokinesis, endocytosis, vesicle transport, and exocytosis. We found that the depletion or null mutation of CTPsyn in border cells resulted in delayed migration in stage 9-10A egg chambers. Moreover, depletion of phosphoinositide kinases also displayed a similar phenotype. Furthermore, double depletion of CTPsyn and phosphoinositide kinases further decreased border cell migration, suggesting that these two pathways were non redundant and that CTPsyn-dependent phospholipid metabolism controls border cell migration. Interestingly, over-expression of either wild-type or enzymatic-dead CTPsyn (CTPsyn^{C399G}) resulted in delayed migration of these cells. Since the PtdInsPs involve in endocytosis and exocytosis, we further investigated the relationship between CTPsyn-mediated phosphoinositide metabolism and endocytosis/exocytosis in this process. Over-expression of Rab11 rescued the migration defect in phosphoinositide kinase deficient cells. Furthermore, downregulation of endocytosis by depletion of Cbl, an ubiquitin E3 ligase, exacerbated the defects in a heterozygous CTPsyn mutant. Furthermore, depletion or deficiency in CTPsyn resulted in loss loss of asymmetric distribution of phosphotyrosine at the leading edge of mutant border cells. However, the aPKC and DE-Cad retained normal patterns in CTPsyn-depleted border cells. In sum, we have define a role of CTPsyn in border cell migration through phosphoinositide metabolism-dependent endocytosis and exocytosis.

353B BMP Regulation Of Germline Development During Testis Stem Cell Niche Formation *Merci Best*, Ashley Fidler, Matthew Wawersik. The College of William & Mary, Williamsburg, VA.

Stem cells are vital for organogenesis, tissue regeneration, and tissue homeostasis. They reside within a stem cell niche and divide asymmetrically to provide the functional cell types necessary for organ function while maintaining a stem cell population that continuously replaces cells lost to death or differentiation. Given their critical role in tissue maintenance, it is important to understanding how these cells first form. To better understand mechanisms regulating stem cell development, we are examining testis stem cell niche formation in *Drosophila melanogaster.* In adult testes, the stem cell niche is comprised of a tightly clustered group of cells which act as a signaling center to regulate sperm-producing germline stem cells (GSCs) as well as somatic cyst stem cells (CySCs). Bone Morphogenetic Protein (BMP) signaling from CySCs to GSCs has also been shown to regulate GSC maintenance in adult and larval gonads (Leatherman & DiNardo, 2010; see also Chang et al, 2013). We have found that BMP signaling shows a dynamic activation pattern in developing germ cells prior to and during testis stem cell niche formation. Here we explore the functional significance of BMP activation during these stages of gonad development. We hypothesize that the BMP signaling pathway functions to repress premature spermatogenetic differentiation and/or germ cell death during testis niche formation. As GSCs are required for continuous gamete production, these studies have implications for organogenesis, fertility, and cancer.

354C Sexual identity in the germline controls niche-germline stem cell communication *Pradeep Bhaskar*, Sheryl Southard, Shekerah Primus, Mark Van Doren. Department of Biology, Johns Hopkins University, Baltimore,MD.

The establishment of sexual identity in the germline is critical for the sex- specific development of germline stem cells and production of sperm vs. eggs. Germ cells depend on signals from the somatic gonad to determine their sex, but in organisms such as flies, mice and humans, the sex chromosome genotype of the germ cells is also important for germline sexual development. When the “sex” of the germline fails to match the “sex” of the soma, germline development and gametogenesis are 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. We have shown that JAK/STAT pathway regulates male identity in the germline. Male somatic cells activate the JAK/STAT pathway in the embryonic germline, while female somatic cells do not. Further, the JAK/STAT pathway is active in both the male and female gonad stem cell niches however, unlike male GSCs, female GSCs do not respond to JAK/STAT pathway. We have found that repression of the JAK/STAT response is dependent on the sex chromosome genotype of the germ cells and the key germline sex determination gene *Sex lethal (Sxl).* Further, regulation of the JAK/STAT pathway is a major function of *Sxl* in the female germline, as oogenesis defects observed

in *Sxl* loss of function can be rescued by also removing JAK/STAT function, including restoration of fertility in a subset of animals. Lastly, *Sxl* regulation of JAK/STAT is important for regulating male germline identity, as either loss of *Sxl* function or activation of JAK/STAT function are sufficient to promote spermatogenesis in XX germ cells when present in testes.

We have also shown that Plant homeodomain finger protein 7 (PHF7) regulates male germline sexual identity and fertility. We show that PHF7 expression is lost when STAT function is blocked in the male germline. This regulation is likely direct, since mutating putative STAT binding sites in *Phf7* promoter eliminates PHF7 expression in the male germline. Since *Sxl* suppresses JAK/STAT in the female germline, PHF7 expression is increased in female GSC's when *Sxl* function is reduced. However, loss of *Phf7* does not rescue the *Sxl* mutant phenotype while loss of STAT does. Thus, there are likely additional important JAK/STAT pathway targets that regulate male germline sexual identity. We propose that an important role of the JAK/STAT pathway is to regulate male identity in the GSCs. Further, intrinsic sexual identity of the GSCs, regulated by *Sxl*, is critical for how GCSs respond to niche signals; female GSCs need to block JAK/STAT signaling in order to retain their proper female identity.

355A Epigenetic Inheritance of Centromeres in Germline Stem cells (EpICS) *Anna A Dattoli*, Ben Carty, Elaine Dunleavy. Centre for Chromosome Biology, Biomedical Sciences Building, NUI Galway.

Background: Stem cells divide asymmetrically generating a self-renewing cell and a second daughter cell programmed to differentiate. The stem-to-differentiation switch, occurring during cell division, is disrupted in common human diseases, including cancer and infertility. Epigenetic mechanisms (which do not alter the primary genomic nucleotide sequence) were recently found to contribute to stem cell maintenance/differentiation. In this context, centromeres are epigenetic chromosomal domains crucial for genomic integrity and accurate chromosome segregation during cell division. Centromeres are specified by the histone H3 variant, CENP-A, assembled in the centromeric nucleosome at the end of mitosis (between telophase and G1), with its loading dependent on the constitutive centromere protein CENP-C and the chaperone/assembly factor HJURP (Holliday Junction Recognition Protein).

Scope: Our aim is to understand whether components of the centromeric chromatin are crucial to maintain the identity of stem cells.

Methodology: We employed quantitative confocal microscopy to identify at what cell cycle phase centromeres are assembled in mother (stem) and daughter (differentiating) cells upon division of germline stem cells (GSCs) in female flies. Following, we used germ cell specific RNAi approaches to verify the influence of specific centromeric components in the commitment of GSCs to differentiation.

Results: Different from other cell types, we show that the assembly of CENP-A in the centromeric nucleosome in both GSCs and daughter cells in female flies occurs during G2 phase. Furthermore, our functional analysis reveals that CAL1 (HJURP homologue in flies) is specifically required for differentiation of the *Drosophila* female GSCs.

Conclusions: Our research identified new elements in the epigenetic pathway that specify and maintain stem cells.

356B Ecdysone signaling controls germline stem cell maintenance by promoting expression of distinct RNA-binding proteins. *Danielle Finger*, Elizabeth Ables. Biology, East Carolina University, Greenville, NC.

Reproductive capacity in many organisms is maintained by the activity of germline stem cells (GSCs), which maintain an undifferentiated fate while creating new daughters destined for differentiation. A complex regulatory network influences stem cell fate, including local signals from adjacent cells and long-range steroid hormonal signals. The molecular mechanisms by which hormone signaling is integrated with the molecular pathways that control cell fate, however, are largely unknown. To elucidate these fundamental mechanisms, we study the role of ecdysone, a prototypical Drosophila melanogaster steroid hormone, in the control of stem cell function. Female GSCs are directly regulated by the steroid hormone ecdysone, which is structurally and functionally similar to human sex hormones. Using a forward genetic screen, we identified Heterogeneous nuclear ribonucleoprotein at 27C (Hrb27C) as a downstream target of ecdysone signaling. Hrb27C is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family of RNA binding proteins, which function in multi-protein complexes to bind nascent mRNAs and regulate their splicing, maturation, and localization. To investigate whether and how Hrb27C regulates GSC maintenance, we used Flippase/Flippase Recognition Target (FLP/FRT)-mediated mosaic recombination to create homozygous Hrb27C mutant GSCs, and used molecular markers to track their fate. We found that Hrb27C mutant mosaics display greater GSC loss than their respective controls, indicating that Hrb27C is required for GSC maintenance. Our data suggest that GSC loss in the absence of Hrb27C is due, at least in part, to deregulation of the BMP signaling pathway that is required for repressing differentiation of GSCs. Moreover, our preliminary data suggest that ecdysone signaling also regulates the expression of other hnRNPs which are also required to maintain the stem cell fate. We are currently testing the hypothesis that Hrb27C, in complex with other hnRNPs, helps maintain stem cell fate in response to ecdysone signaling by stabilizing the expression of transcripts critical for the stem cell fate. Given the similarity between Drosophila and human steroid hormone signaling, and the importance of post-

transcriptional control in cell fate decisions, our study will help clarify how stem cell activity is tethered to changes in the physiological environment, and provide new insight into the molecules critical for this response.

357C A transcriptomic approach to identify new genes required for Germline Stem Cell maintenance in *Drosophila melanogaster* *M. Lobo-Pecellín*, A. González-Reyes. Andalusian Center for Developmental Biology, Pablo de Olavide University, Carretera de Utrera km.1, 41013 Seville, Spain.

Stem cell activity must be strictly regulated to ensure a proper balance between proliferation and differentiation. This regulation is possible because stem cells reside in specific and restricted microenvironments called niches. The *Drosophila* ovary develops well-defined niches that contain on average 2-3 germline stem cells (GSCs). GSC maintenance depends on systemic and local factors. A known player in the decline of stem cell populations is the age of the animal. Thus, 4-week old females show a 50% decrease in the number of GSCs per niche when compared to young flies. In order to identify new niche factors affecting stem cell loss in aged niches, we have performed a transcriptomic analysis of niche cells of progressively older ovaries and compared them to younger controls. Our studies have identified 63 genes whose expression is either up-regulated (30) or down-regulated (33) in aged niches. To validate functionally the putative candidates, we have overexpressed or used RNAi of candidate genes in niche cells. While the characterisation of presumptive targets is undergoing, our approach has already yielded a group of genes with a putative role in GSC maintenance. We will present our current efforts aimed at defining the ageing of the *Drosophila* ovarian germline stem cell niche at the genetic level.

358A Signaling via a G-protein coupled receptor increases male germline stem cell division frequency in response to mating. *Manashree Malpe*[1], Benjamin Parrot[2], Karl Kudyba[1], Leon McSwain[1], Vinay Choksi[3], Chun Ng[1], Alicia Hudson[1], Cordula Schulz[1]. 1) Cellular Biology, University of Georgia,Athens, Athens, GA; 2) Odum School of Ecology,University of Georgia,Athens, Athens, GA; 3) Duke University School of Medicine, Durham, NC.

Adult stem cells are precursors for specialized cells in the body and of wide interest to research in biology and medicine. The stem cell microenvironment or niche is important for specifying and maintaining the stem cell populations. However, the physiological and developmental state of an organism plays an important role in stem cell activity.

Stem cell activity is measured as a rate of their division frequency. We present a detailed analysis revealing that male germline stem cell (GSC) division frequency ranges widely within wildtype animals. We further show that males that were heavily mated to virgin females always displayed a significant increase in their germline stem cell (GSC) division frequency compared to their non-mated siblings. Mated wildtype females did not increase their GSC division frequency in response to mating and the number of GSCs in the testes or the ovaries of wildtype animals was not affected by mating.

To study the increase in male GSC division frequency at a molecular level, our lab uses the UAS-Gal4 expression system in combination with RNA-interference. Knockdown of G-alpha-i or G-gamma-1 from the germline cells abolished the increase of GSC division frequency in mated males indicating that GSC division frequency is regulated by the activity of at least one G-protein coupled receptor. We will present data indicating the nature of this receptor.

359B The role of H1 in germline stem cells maintenance *Jianquan Ni*. School of Medicine, Tsinghua University, Beijing, China.

Epigenetics plays critical roles in controlling stem cell self-renewal and differentiation. Histone H1 is one of the key chromatin regulators, but its role in adult stem cell regulation remains unclear. One of the major challenges in elucidating the developmental roles of H1 is its high copy number. We have applied the transgenic RNAi technique to efficiently knockdown H1 in the *Drosophila* germline. This approach has revealed that H1 is intrinsically required in the regulation of GSCs. Depletion of H1 in GSCs resulted in their premature differentiation through activation of the key GSC differentiation factor bam. In addition, the acetylated H4 lysine 16 (H4K16ac) is selectively augmented in the H1-depleted GSCs. Furthermore, overexpression of mof reduces H1 association on chromatin. Most interestingly, depletion of mof significantly rescues the GSC loss phenotype. Taken together, these observations suggest that H1 functions intrinsically to promote GSC self-renewal by antagonizing MOF function. Since H1 and H4K16 acetylation are highly conserved between flies to humans, our discoveries in flies might be applicable to stem cells in higher organisms. For example, dysregulated H1 has been reported to contribute to ovarian tumorigenesis in humans. Interestingly, we have recently noticed that when H1 is specifically knocked down in the escort cells (ECs) in the germaria, which form part of the niche for the differentiation of the GSCs and cystoblasts, an increase of GSC-like, spectrosome-containing cells in the germaria was observed, which resembles an ovarian tumor.

360C Wuho controls mitosis accuracy of germ cells for meiosis entry in drosophila females *Elham Rastegari*[1,2], Tao-shih Hsieh[1,2], Hwei-Jan Hsu[3]. 1) Molecular and Cell Biology Program, Taiwan International Graduate Program,National Defense Medical Center and Academia Sinica, Taipei 115, Taiwan; 2) National Defense Medical Center and Academia Sinica; 3) Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 115,

Taiwan.

Abscission is the final event of cell division that physically separates the cytoplasm of sister cells; however, how it is regulated remains unclear. Wuho (Wh), a WD40 repeat protein, maintains genome stability by promoting DNA replication from fly to human; deletion of WH causes apoptosis in cultured cells. Although *wh* mutant females are sterile, only a few proportion of *wh* mutant germ cells undergo apoptosis, suggesting additional roles of WH in the control of oogenesis. Here, we report that Wh promotes abscission in GSCs but prevents complete division of germ cells in a cyst. Loss of Wh delays abscission in GSCs, therefore GSCs cannot separate from their progeny (stem-cysts). In contrast, depletion of Wh promotes abscission in germ cell cysts results in gem cells apart from a cyst. Such defect may lead to asynchrony division of germ cells and therefore produces odd number of germ cells, instead of 16 cells in a wild-type cyst in which meiosis is initiated. As a consequence, *wh* mutant cells are failed to enter meiosis, revealed by the absence of synaptonemal complex and meiosis-induced double-strand break. Furthermore, *wh* mutant GSCs are quickly lost and exhibit a prolonged S phase. Interestingly, similar phenomena are not observed in males, indicating a sex specific role of Wh. In summary, Wh maintains the GSC pool, and controls germ cell mitosis by promoting S phase progression and ensuring accurate abscission. Here, we uncover a novel role Wh during cytokinesis, and that may be evolutionary conserved across species.

361A Characterizing Chinmo: Structure-Function Analysis of a Stem Cell Sex-Maintenance Factor *Leanna Rinehart*, Oliver Kerscher, Matthew Wawersik. The College of William & Mary, Williamsburg, VA.

Assignment of cells' sex is essential for a species to retain sexual dimorphism and the ability to reproduce sexually. In many organisms, sex-specific transcriptional (or cellular) programs must also be maintained throughout an organism's lifetime. For instance, if sex of stem cells is not continually reinforced, then entire organs could potentially switch sex (Zarkower, 2014). Chronologically inappropriate morphogenesis (Chinmo) is a putative transcription factor of the BTB-Zinc finger family found in *Drosophila* that regulates cell fate and behavior, and is essential for maintenance of male stem cell sex in the testis. Previous research indicates that Chinmo prevents feminization of stem cells in the adult male gonad and is, therefore, associated with male characteristics in fruit flies (Ma et al., 2014). Goals of this research are to investigate how Chinmo is regulated and how Chinmo controls cell fate and behavior in the fruit fly *Drosophila melanogaster*. We hypothesize that key domains and modifiers of Chinmo, including SUMO (Small Ubiquitin-Like Modifier), modulate Chinmo localization and function. In our preliminary analysis, we found that Chinmo contains several putative SUMO interacting motifs (SIMs) and sumoylation consensus sites that may play a role in regulating its function. Using two-hybrid assays we identified several Chinmo interacting proteins, and are investigating their functional relevance in the *Drosophila* testis using RNAi knockdown. Subsequent studies are aimed at identifying domains of Chinmo that may control its subcellular localization. In summary, this research elucidates the function of the *Drosophila* protein Chinmo and sheds light on how stem cell sex is properly regulated throughout an organism's lifetime.

362B Thickveins, a BMP receptor, determines the Size of the Primordial Germ Cell Pool via the Soma through a Non-Canonical BMP signaling *Chen Yuan Tseng*, Yu Han Su, Hwei Jan Hsu. Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan.

Germline stem cells (GSCs) resided in testes, and often in ovaries, continuously produce germ cells that eventually develops into sperms and oocytes. GSCs arise from a subset of primordial germ cells (PGCs), which are intermingled and regulated by gonadal somatic cells(soma). During development, PGCs expend their number and then undergo differentiation to initiate gamatogenesis, except those are selected as GSCs. However, the regulatory mechanisms involved in these processes are largely unclear. Here, we report that Thickveins (Tkv), an ortholog of mammalian type I TGFβ receptor of BMPs, functions in the soma to control the size of the PGC pool for ovary fitness. Knock down *tkv* in the soma throughout development results in female sterile, due to the differentiation program of PGCs is completely disrupted. Stage-dependent knock down experiments further suggest that Tkv limits PGC proliferation before third-instar larval (L3) stage, while promotes PGC differentiation after L3 stage. Interesting, suppression of BMP downstream components do not result in a similar phenotype, suggesting Tkv exert its function in the soma via a non-canonical BMP signaling pathway. Instead, Tkv-mediated signaling interacts with Egfr signaling in the soma, probably in parallel or upstream of Wnt6 to regulate PGC proliferation. Tkv also coordinates with Hh and Egfr signaling to promote PGC differentiation. It is still not clear how Tkv switches its function from limiting PGC proliferation to promote PGC differentiation, how Tkv interacts with Wnt6, Egfr and Hh signaling to control PGC proliferation or differentiation, and what signals activates Tkv in the soma. Nevertheless, our finding has discovered a novel role of BMP receptor in maintaining of a proper size of the PGC pool.

363C Control of germline stem cell differentiation by Polycomb and Trithorax group genes in the niche microenvironment. *Fu Yang*, Xuewen Li, Xinghua Li, Bowen Deng, Rongwen Xi. National Institute of Biological Sciences, Beijing, Beijing, China.

Polycomb and Trithorax group (PcG and TrxG) genes function to regulate gene transcription by maintaining a

repressive or active chromatin state, respectively. This antagonistic activity is important for body patterning during embryonic development, but whether this function module has a role in adult tissues is unclear. Here, we report that in the Drosophilaovary, disruption of the Polycomb repressive complex 1 (PRC1), specifically in the supporting escort cells, causes blockage of cystoblast differentiation and germline stem celllike tumor formation. Tumors are caused by derepression of decapentaplegic (dpp), which prevents cystoblast differentiation. Interestingly, activation of dppin escort cells requires the function of the TrxG genebrahma(brm), suggesting that loss of PRC1 in escort cells causes Brm-dependentdppexpression. Our study suggests a requirement for balanced activity between PcG and TrxG in an adult stem cell niche, and disruption of this balance could lead to the loss of tissue homeostasis and tumorigenesis.

364A Drosophila intestinal stem cells express multiple autocrine and paracrine ligands to maintain homeostasis *David Doupé*[1], Owen Marshall[2], Andrea Brand[2], Norbert Perrimon[1,3]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK; 3) Howard Hughes Medical Institute, Boston, MA.

Epithelial homeostasis requires the precise balance of epithelial stem / progenitor cell proliferation and differentiation. While many of the signaling pathways that regulate epithelial stem cells have been identified, less is known about their targets or crosstalk between them. Here we use gene expression profiling by targeted DamID to identify the stem / progenitor specific transcription and signaling factors in the Drosophila midgut. Many signaling pathway components, including ligands of most major pathways, exhibit stem / progenitor specific expression and have regulatory regions bound by both intrinsic and extrinsic factors. In addition to previously identified stem / progenitor-derived ligands we show that both the insulin-like peptide Ilp6 and the TNF ligand eiger are specifically expressed in the stem / progenitors and regulate normal tissue homeostasis. We propose that the integration of extrinsic and cell-type specific regulatory factors allows stem and progenitor cells to maintain a homeostatic micro-environmental niche through the expression of autocrine and paracrine factors.

365B The Chromatin Remodeling Factor Kismet/CHD7 Controls Intestinal Stem Cell Proliferation *Louis Gervais*[1], Carolina Perdigoto[1], Owen Marshall[3], Andrea Brand[2], Francois Schweisguth[4], Allison Bardin[1]. 1) Institut Curie, CNRS - INSERM , 26 rue d'Ulm 75005 Paris - France; 2) Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom CB2 1QN; 3) Menzies Institute for Medical Research Medical Science Precinct 17 Liverpool Street Hobart TAS 7000 Australia; 4) Institut Pasteur, 25-28 Rue du Docteur Roux 75015, Paris France.

Replacement of specialized cells occurs in most adult tissues and relies on the activity of resident adult stem cells. Increasing evidence highlights the importance of the regulation of chromatin structure both in the maintenance of stem cell identity and in their commitment to differentiation, yet, therole of such regulation in adult stem cells in vivo is poorly understood. Using the *Drosophila* intestine as a model to identify new factors regulating tissue homeostasis and adult stem cell activity, we found that clonal inactivation of the chromatin remodeling factor Kismet results in a strong increase in relative number and density of intestinal stem cells (ISC) in the gut. This is due to an abnormal division rate of *kismet* mutant stem cells compared to wild-type stem cells. Kismet is a member of the chromodomain-helicase-DNA binding (CHD) subfamily of ATP-dependent chromatin remodeling factors. CHD proteins are thought to function in the nucleus via binding to the DNA and thus regulate gene transcription. Mutations in CHD7 are a common genetic cause of CHARGE Syndrome. Although most of the disorders affecting individuals with CHARGE are due to defects during embryonic development, recent published work points toward essential functions of CHD7 in adult tissues. To understand Kismet's role in adult stem cells, we established the genes bound by Kismet in the ISC using genome wide mapping of Kismet protein with targeted DamID. Our findings suggest that aberrant activation of EGFR and Jak/Stat pathways occurs in *kismet* mutant driving stem cell overproliferation. We propose that Kismet is a major regulator of ISC proliferation networks, required for correct tissue homeostasis.

366C A New Bioinformatics Tool to Integrate Data from Genome-wide Screens into Cytoscape Networks *Armen Halajyan*, Calvin Apodaca, Mariano Loza-Coll. Department of Biology, California State University, Northridge - Northridge, CA.

The homeostasis of many of our organs is sustained by the division of adult (or tissue) stem cells. Consequently, defects in stem cell proliferation and/or differentiation can lead to disease. While we have a fairly good understanding of which genes promote stem cell self-renewal or differentiation, we still have a relatively poorer understanding of the genetic mechanisms coordinating a stem cell’s choice between these fates. Over the years, and as high-throughput technologies have become increasingly cheaper and easier to use, several research groups have obtained genome-wide data related to a number of key regulatory factors in diverse *Drosophila* stem cell types. Most frequently, these datasets are in the form of a simple list of targets or “hits” that correspond to differentially expressed genes, protein interactors, binding sites in DNA, etc. We have developed a simple and intuitive computer program that allows users to integrate several such datasets into files that can later be used with the Open Source software Cytoscape to visualize and analyze small networks and modules that originate when shared targets are linked across the combined

datasets. Our program also allows users to import protein and genetic interaction data from FlyMine (and other Intermine-based data repositories) to enrich the number of shared interactions. We have used our program to combine three independent datasets related to putative targets of three key regulators of intestinal stem cells, Capicua (Cic), Stat92E and Escargot (Esg), and identified a subset of 47 genes that are potentially regulated by all three factors. Interestingly, this set of genes was found to be significantly enriched for genes highly expressed in progenitor cells of the posterior midgut. In summary, we have developed a simple tool that will allow experimental biologists with minimal bioinformatics experience to combine several large datasets into network maps that can facilitate the identification of interesting candidates for further studies.

367A Mitochondrial Pyruvate Metabolism Suppresses Stem Cell Proliferation *Dona Wisidagama*[1], John Schell[2], Jared Rutter[2], Carl Thummel[1]. 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) Biochemistry Department, University of Utah, Salt Lake City, UT.

Metabolism is thought to provide a permissive metabolic state that supports glycolysis and biosynthetic pathways in stem cell proliferation. Here we show that metabolism can also play a direct and active role in stem cell function through genetic studies of the Mitochondrial Pyruvate Carrier (MPC) in *Drosophila* and mice. The MPC is necessary and sufficient for mitochondrial pyruvate import in yeast, *Drosophila* and mammals, linking cytoplasmic glycolysis with mitochondrial oxidative phosphorylation. This placement of the MPC provides a unique opportunity to modulate cytoplasmic pyruvate entry into mitochondria. Using mutant clonal analysis and cell-specific RNAi we show that the MPC is required in a cell autonomous manner to suppress excess proliferation in *Drosophila* intestinal stem cells (ISCs). Similar results were obtained when MPC function was disrupted in LGR5-expressing ISCs in mice. In addition, we observed that, in the absence of the MPC, differentiated enterocytes activate an inflammatory response and secrete the cytokine Unpaired-3 (UPD3). In *Drosophila*, UPD3 ligand activates JAK/STAT signaling in ISCs thereby enhancing their proliferation. Our studies thus demonstrate that the MPC is required in both a cell-autonomous and non-autonomous manner to control ISC proliferation. This work reveals that mitochondria play an active role in allowing ISCs to maintain their proliferation and homeostasis by sensing alterations to their own energy state and that of neighboring differentiated cells.

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368B Post-transcriptional regulation of Ttk69 by Sina and Phyl promotes enteroendocrine cell specification *Chang Yin*[1,2], Rongwen Xi[1]. 1) National Institute of Biological Sciences, Beijing, China; 2) Graduate Program, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China.

The intestinal epithelium in the *Drosophila* midgut provides a simple and genetically tractable system for understanding stem cell regulation and cell fate determination during multiple cell lineage differentiation. The resident multipotent intestinal stem cells (ISCs) can self-renew and generate progenitor cells that differentiate into either absorptive enterocytes (ECs) or secretory enteroendocrine cells (EEs). We have recently identified Ttk69 as a master repressor of EE specification in *Drosophila* midgut. Loss of Ttk69 causes all progenitor cells to adopt EE cell fate. But how Ttk69 is regulated to control EE specification is unknown. In *Drosophila* eye and external sensory organ development, Ttk69 is regulated by E3 ubiquitin ligase Sina and its adaptor protein Phyl for R7 and SOP cell specification. We found that in *Drosophila* intestine, Sina and Phyl are required for EE specification. Similar to the loss of *ttk69*, overexpression of Phyl induces supernumerary EE cells. Our genetic and cellular analyses suggest that Sina and Phyl functions upstream of Ttk69 to regulate Ttk69 stability.

Our study therefore demonstrates an important role for the neural Sina-Phyl-Ttk pathway in regulating EE specfication in adult Drosophila midgut.

369C Regulation of crystal cells by Activinβ signaling and neuronal activity *Itrat Batool*[1], Katelyn Kukar[1], Murray Andrews[1], Katja Brueckner[1,2,3]. 1) Department of Cell and Tissue biology; 2) Broad Center of Regeneration Medicine and Stem Cell Research; 3) Cardiovascular Research Institute, University of California San Francisco, CA.

Drosophila blood cells, or hemocytes, can differentiate into three distinct cell types: macrophage-like plasmatocytes, crystal cells and lamellocytes. Previous work has shown that the survival, localization and proliferation of resident embryonic-lineage plasmatocytes in the *Drosophila* larva depends on the peripheral nervous system (PNS) in the hematopoetic pockets (HPs) of the *Drosophila* larva. Specifically, Activinβ (Actβ), a TGF-β family ligand, plays a role in the neuron-activity dependent promotion of plasmatocyte proliferation. Recent reports have shown that embryonic-lineage plasmatocytes in the *Drosophila* larva have plasticity to differentiate into crystal cells and lamellocytes, suggesting that at least a fraction of plasmatocytes is oligopotent. Since the regulation of these transitions remains incompletely understood, we examined whether Actβ signaling in plasmatocytes promotes the development of crystal cells. Indeed, we find that RNAi silencing of Actβ/dSmad2 pathway signaling in plasmatocytes,

but not in differentiated crystal cells, leads to a dramatic decrease in the number of crystal cells. Conversely, Actβ overexpression in PNS neurons increases the crystal cell count per larvae. Moreover, specific silencing of sensory neurons by transient expression of the inward rectifying potassium channel *Kir2.1* dramatically reduces crystal cell number. Crystal cells typically cluster in the terminal segment of the larva, preferentially in the hematopoietic pockets comprising neurons of the terminal sensory cones, suggesting dependence on these microenvironments. Since manipulations of Actβ/dSmad2 signaling or silencing of PNS neurons generally show more dramatic effects on crystal cells than on plasmatocyte numbers, our findings suggest a role for Actβ signaling and neuronal activity in plasmatocyte-to-crystal cell differentiation. Current research examines this hypothesis and the role of Actβ signaling and PNS neuron activity in various other aspects of crystal cell development and homeostasis, drawing potential parallels with independent self-renewing blood cell populations in vertebrate systems.

370A Transcriptional changes in *Drosophila* hemocytes following cholinergic stimulation *Leire Herboso*[2], Itrat Batool[2], Corinna Wong[2], Katja Brückner[1,2,3]. 1) Broad Center of Regeneration Medicine and Stem Cell Research Cell and Tissue, UCSF, San Francisco; 2) Dept. Cell and Tissue Biology; 3) Cardiovascular Research Institute, University of California San Francisco, CA.

In the *Drosophila* larva, the peripheral nervous system (PNS) interacts with the blood cell system of hemocytes in specific microenvironments known as Hematopoietic Pockets (HPs). In the HPs, hemocytes of the embryonic lineage are in direct contact with sensory neuron clusters of the PNS, which promote hemocyte proliferation, survival and localization; these hemocytes show close parallels with the independent myeloid system of self-renewing tissue macrophages in vertebrates. *Drosophila* sensory neurons of the PNS are cholinergic, i.e. they use acetylcholine as a major neurotransmitter. Previously, we found that stimulation by the pan-Acetylcholine Receptor (AChR) agonist carbachol facilitates recruitment of hemocytes to the HPs and promotes proliferation and increase in the total number of hemocytes per larva, while PNS specific silencing by transient expression of the inward-rectifying potassium channel Kir2.1 has opposite effects. Moreover, previous work in the lab identified Activinβ (Actβ), a TGF-β family ligand, as one of the key factors in neuron-dependent hemocyte regulation.

Seeking to identify transcriptome changes in hemocytes under conditions of neuronal stimulation and varying levels of Activinβ signaling, we took a RNAseq approach. Specifically, we analyze changes induced in *Drosophila* hemocytes after stimulation with the pan-AChR agonist carbachol, or RNAi silencing of Actβ/dSmad2 signaling. In parallel, our goal is to understand transcriptional changes in embryonic-lineage hemocytes over the course of larval development. Differentially regulated genes identified in these assays will provide a better understanding of the hemocyte responses induced by PNS activity, and will suggest hemocyte genes that may also be regulated by upstream sensory stimuli from the environment, thereby linking environmental inputs with the adaptation of the blood cell pool.

371B Exploring cell type specific gene expression profiles within the adult *Drosophila* gonads *Miriam Akeju*[1], Justin Fear[2], Brian Oliver[2], Erika Matunis[1]. 1) Dept. of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Stem cell self-renewal and differentiation are complex processes that require stem cells to maintain their identity and also respond to changes within their microenvironment, the stem cell niche. Understanding how stem cell fate is regulated within stem cell niches is fundamental to expanding our knowledge of tissue regeneration. Previous studies identified the Jak/STAT signaling effector chinmo as a gene that plays a critical role as a stem cell fate determinant (Flaherty et al., *Dev Cell*, 2010). Using the Drosophila testis stem cell niche as a model system, chinmo was shown to have another important role: it actively maintains male sex identity in adult somatic stem cells of the testis by preventing the feminization of these cells (Ma et al., *Dev Cell*, 2014). Identifying the downstream targets of chinmo that maintain stem cell fate and/or sex identity is crucial to further understand how stem cell fate is modulated. To first gain a better understanding of the normal genomic expression patterns of stem cells within the niche, we performed Targeted DNA Adenine Methylation Identification on the somatic stem cells in both the testis and ovary allowing us to gain insight on the expression patterns in this specific subset of cells. These results will allow us to identify novel genes expressed specifically within male and female somatic stem cells. This method can also be modified to allow us to study the gene expression patterns of the somatic stem cells under aberrant conditions such as during the loss or overexpression of Chinmo within the testis or ovary, respectively. This work has the potential to significantly advance our understanding of how stem cell fate and sex identity is controlled mechanistically.

372C Cell-Cell Adhesion Controlled by Hedgehog Signaling Establishes Precursors for Germline Stem Cell Niches *Chun-Ming Lai*[1,2,3], Kun-Yang Lin[1,2,3], Shih-Han Kao[3], Yi-Ning Chen[5], Fu Huang[6], Hwei-Jan Hsu[1,3,4]. 1) Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, Academia Sinica and National Chung-Hsing University, Taipei, 11529, Taiwan; 2) Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan; 3) Institute of Cellular and Organismic Biology, Academia Sinica; 4)

Biotechnology Center, National Chung-Hsing University, Taichung, 40227, Taiwan; 5) Institute of Molecular and Cell Biology, Academia Sinica; 6) Institute of Biological Chemistry, Academia Sinica, Taipei, 11529, Taiwan.
Stem cells require different types of supporting cells, or niches, to control stem cell maintenance and differentiation. However, little is known about how those niches are formed. Here, we report that in the development of the *Drosophila* ovary, the anterior-posterior Hedgehog (Hh) signaling gradient sets differential cell affinity for somatic gonadal precursors to specify stromal intermingled cells, which contribute to both germline stem cell maintenance and differentiation niches in the adult. We also report that Traffic Jam (an ortholog of a large Maf transcription factor in mammals) is a novel transcriptional target of Hh signaling to control cell-cell adhesion by its negative regulation of E-cadherin expression. Our results demonstrate the role of Hh signaling in niche establishment via the control of cell-cell affinity to segregate somatic cell lineages for differentiation. Such morphogen-guided signaling may be generally used in other organs to generate stem cell niches.

373A A Switch in Cortical Binding Mode is Essential for Asymmetric Segregation of Fate Determinants in *Drosophila* neural stem cells. *Matthew R Hannaford*, Anne A Ramat, Nicolas Loyer, Jens Januschke. School of Life Sciences, University of Dundee, Dundee, Scotland, United Kingdom.
The asymmetric segregation of fate determinants upon cell division is an evolutionary conserved mechanism to generate cell fate diversity. A widely used model for this are the self-renewing asymmetric neuroblast divisions of the developing *Drosophila* central nervous system. Neuroblasts polarize in an apical – basal manner localizing the conserved Par complex to the apical cell cortex and then a series of fate determinants such as Miranda to the basal cell cortex, segregating them into one of the two daughter cells, promoting differentiation. Much genetic analysis has gone into identifying the mechanisms by which Miranda polarization is achieved. It has been demonstrated that direct phosphorylation of Miranda by the apically localized kinase aPKC is essential for excluding Miranda from the apical domain by preventing direct membrane binding(1), while Myosins are also required for cortical attachment(2), however these mechanisms have not yet been successfully integrated. By combining live cell imaging of primary neuroblast cultures expressing endogenous reporters for the components of this system, gene editing and pharmacological inhibition we find neuroblast polarization is a highly dynamic process. We reveal that Miranda is bound to the plasma membrane independently of actin and myosin through interphase via a previously identified basic and hydrophobic motif(3), and that this binding is regulated by aPKC. However, upon nuclear envelope breakdown Miranda switches binding mode, relying on Actin and Myosin activity to anchor it to the basal cell cortex. Furthermore, through FRAP analysis of cortical Miranda in control and perturbed conditions, we demonstrate that Miranda binding kinetics vary. In summary, we show that two, temporally distinct mechanisms control the establishment and maintenance of Miranda asymmetry. We therefore believe that direct phosphorylation of fate determinants by aPKC and the role of Actin and Myosin can be integrated into one model.
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374B Investigating Mechanisms of Asymmetric Cell Division and Epigenetic Inheritance Patterns at Specific Genomic Loci *Elizabeth Kahney*, Matthew Wooten, Rajesh Ranjan, Lijuan Feng, Xin Chen. Biology, Johns Hopkins University, Baltimore, MD.
Stem cells rely on epigenetic mechanisms to regulate cell identity as they undergo **asymmetric cell division (ACD)** to give rise to a daughter that self-renews and another daughter that differentiates, despite each containing identical genomes. Pioneering work from our lab developed a dual-color labeling system to study ACD in *Drosophila* male **germline stem cell (GSCs)** and found that pre-existing (old) histones are preferentially retained in the self-renewed GSC while newly synthesized (new) histones are enriched in the differentiating daughter. Histone modifications play important roles in regulating gene expression and thus stem cells may maintain their identity by selectively inheriting pre-existing histones. In contrast to the global histone asymmetry seen between the daughters of a male GSC division, histone inheritance patterns in actively dividing female GSCs appear symmetric. However, correlation mapping can identify significant regions of non-overlapping old and new histone signal suggesting that asymmetric histone inheritance may be occurring at more specific, local regions of the genome. **Hypothesis:** During ACD, the self-renewing daughter inherits old histones with modifications that maintain active expression at stem cell-promoting genes and repression at differentiation genes. This allows for continuation of the stem cell state by maintaining epigenetic memory. Meanwhile, the differentiating daughter inherits new histones in these regions, erasing the epigenetic stem cell memory and allowing for differentiation to occur.
To explore the possibility of local histone asymmetry occurring at genomic regions that undergo expression changes during GSC division, I have created an array of fluorescent DNA oligopaint probes targeting well-

characterized genes associated with promoting the stem-cell state or the differentiation program such as *daughters against Dpp* and *bag of marbles*, respectively. By visualizing these probes along with antibodies against old versus new histones and histone modifications in a combined immunofluorescence labeling and fluorescent *in situ* hybridization (IF-FISH) approach, I am elucidating changes in the epigenome that occur during ACD. Furthermore, I am investigating the mechanisms that allow the mitotic machinery to "read out" the asymmetric chromatin and appropriately segregate the sister chromatids. Specifically, preliminary data suggests temporal asymmetries in the condensation and phosphorylation of the chromatin paralleled with centrosome and microtubule activity may underlie the asymmetric attachment and segregation of sister chromatids preferentially containing old versus new histones.

375C Ribosomal DNA sequence is required for nonrandom sister chromatid segregation during asymmetric division of *Drosophila* male germline stem cells *George J. Watase*[1,2], Yukiko M. Yamashita[1,2,3]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; 3) Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI.

Sister chromatids are the product of DNA replication, thus are thought to be an identical copy of each other. Despite this, we previously reported that, in *Drosophila* male germline stem cells (GSCs), sister chromatids of X and Y chromosomes, but not autosomes, are segregated nonrandomly between a self-renewing stem cell and a differentiating daughter, suggesting that sister chromatids may carry distinct information (Yadlapalli and Yamashita 2013). However, the underlying mechanism as well as potential distinct information carried by two sister chromatids remain unknown. To begin address these questions, we first attempted to identify chromosomal region that are required for nonrandom segregation. We found that one of the X heterochromatin-deletions, *bb*^{158}, which lacks entire rDNA locus and a large portion of the 359-bp satellite repeat showed a random segregation of X chromosome sister chromatids. As there is no X chromosome deletion strains were available that distinguish rDNA vs. 359-bp satellite repeats, we took advantage of Y chromosome deletion that lacks rDNA locus: whereas both X and Y chromosomes contain rDNA locus, 359-bp repeats are present only on X. *Ybb*$^{-}$, which lacks rDNA locus on Y chromosome, resulted in random segregation of Y chromosome sister chromatids. These results indicate that rDNA loci may be responsible for nonrandom sister chromatid segregation. This is also consistent with the observation that only X and Y chromosomes, but not autosomes, display nonrandom sister chromatid segregation. Each rDNA locus consists of about 200-250 copies of rDNA unit, containing rRNA genes (18S, 5.8S, 28S), ETS (external transcribed spacer), ITS (internal transcribed spacer) and IGS (intergenic spacer). Interestingly, it has been known that *Drosophila simulans*, a sibling species of *D. melanogaster*, has repeats that only contain IGS sequence (but not rRNA genes, ETS and ITS) on its Y chromosome, and we found that the sister chromatids of Y chromosome in *D. simulans* are segregated nonrandomly. These results suggest that IGS sequence may be responsible for non-random sister chromatid segregation. It awaits future investigation how mechanistically IGS sequence leads to nonrandom sister chromatid segregation and for what purpose(s) IGS-mediated nonrandom sister chromatid segregation serves. **Reference:** Yadlapalli, S., and Y. M. Yamashita, 2013 Chromosome-specific nonrandom sister chromatid segregation during stem-cell division. Nature 498**:** 251-254.

376A A Tunable Rate of Commitment Enables Stem Cell Scaling During Resizing of Adult Organs *XinXin Du*[1,2], Lucy O'Brien[1], Ingmar Riedel-Kruse[2]. 1) Molecular and Cellular Physiology, Stanford University, Stanford, CA; 2) Bioengineering, Stanford University, Stanford, CA.

Many adult organs grow or shrink to accommodate fluctuating levels of physiological demand. As total cell number changes, stem cell number changes proportionally. This stem cell scaling likely enables tissue homeostasis throughout a broad range of organ sizes. However, the cellular behaviors that give rise to scaling are unknown. Here we study two complementary biophysical models of the adult Drosophila midgut, one of the best-understood examples of stem cell scaling. These models recapitulate the physiological kinetics of scaling during midgut growth and shrinkage, but only if the rate of terminal fate commitment is tuned to the existing proportion of stem cells. Furthermore, tunable commitment can be explained by the concept of a 'stem cell territory', i.e., the physical space that a stem cell explores during its lifetime. Scaling requires a threshold territory size, which in turn depends on physical cell properties such as cell-cell adhesion, motility, and fate signals. These models provide a conceptual basis to understand stem cell scaling in general and lead to the prediction that scaling requires a tunable commitment rate.

377B A novel role for Plexin A in photoreceptor axon guidance *Jessica Douthit*, Sergio Astigarraga, Gina Lee, Jessica Treisman. Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY.

Normal nervous system functioning is dependent upon very defined and precise network formation during development. Aberrant synaptic connectivity caused by mutations in axon guidance molecules and cell adhesion proteins has been associated with neurodevelopmental and psychiatric disorders such as intelligence disability,

epilepsy, Autism Spectrum Disorders, and schizophrenia. The *Drosophila* visual system is an excellent model system for studying the basic mechanisms of axon pathfinding and neural circuit formation. The terminals of R7 and R8 photoreceptors, responsible for color vision, are segregated into distinct target layers of the medulla, a central region of visual processing in the brain. We have found that null mutations in *plexA* cause R7 photoreceptors to prematurely terminate in the R8 layer of the medulla and fail to expand their axon terminals. Labeling of presynaptic sites in R7 axons shows fewer synapses when *plexA* is knocked down in neurons using RNAi. Mosaic analysis and *plexA* RNAi experiments indicate that PlexA is required in the brain and not in the eye. PlexA is strongly expressed in medulla tangential neurons, which occupy the layer just beyond where R7 axons terminate and arise from the tips of the outer proliferation center (OPC). Deleting *plexA* from the progeny of the tips of the OPC using somatic CRISPR/Cas9 causes R7 mistargeting, supporting a function for PlexA in medulla tangential neurons. Misexpression of PlexA in photoreceptor axons results in their hyperfasciculation and premature termination, consistent with PlexA acting to promote attraction or adhesion of R7 axons. R7 mistargeting is not observed in mutants for Semaphorin-1a or Semaphorin-1b, the known PlexA binding partners. One reported splice form of PlexA lacks the Semaphorin-binding Sema domain. We are using misexpression and genome editing to test whether the Sema domain is necessary for PlexA function in the visual system. We are also deleting the cytoplasmic domain in order to determine whether PlexA acts as a receptor or a ligand in this context. Lastly, we are investigating whether a novel receptor may mediate this function of PlexA, which may have implications for how Plexin family members control axon pathfinding in higher organisms.

378C Dscam1 signaling and function in growth cones. *A. Izadifar*[1,2], Y. Kise[3], O. Urwyler[4], M.N. Özel[5], E. Agi[5], P.R. Hiesinger[5], D. Schmucker[1,2]. 1) Center for Human Genetics, University of Leuven, Leuven, Belgium; 2) Neuronal Wiring Laboratory, Center for Biology of Disease, VIB, Leuven, Belgium; 3) Department of Biological Sciences, University of Tokyo, Tokyo, Japan; 4) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland; 5) Institute for Biology, Division of Neurobiology, Freie Universitaet Berlin, Berlin, Germany.

In *Drosophila* mechanosensory neurons (ms-neurons), the loss of Dscam1 results in striking axon branching defects, where the axons can reach the CNS and initiate branching, but those branches become aberrantly entangled and collapse. Furthermore, neurons with normal levels of Dscam1 yet an experimentally reduced repertoire of isoforms show normal axon growth. Strikingly, however, these axons cannot form any axon collaterals. Currently, we are investigating how Dscam1 controls axon branching molecularly and what effector pathways underlie its isoform-independent as well as isoform-dependent functions.

We performed an *in vivo* reverse genetic screen to identify Dscam1-controlled effector proteins and generally factors involved in axon collateral formation. We primarily focused on actin regulators, adaptors, kinases, phosphatases and candidates identified in biochemical screens (e.g. SCAR, Wave complex, Dock, Ena, Msn, and Tao1).

Our results suggest an antagonistic role of Dscam1 and actin regulators in axonal branching. For example, increasing levels or activity of positive promoters of actin polymerization (Wave) lead to Dscam1 loss-of-function phenotypes in mechanosensory axons. We further find evidence suggesting an important role for msn and tao1 in restricting the formation of axon collaterals. We will present genetic and biochemical results suggesting that Dscam1 via Msn and Tao1 can locally and likely directly control actin regulatory molecules in sprouting growth cones.

Moreover, to improve our ability to analyze dynamic cytoskeletal processes underlying axon branching we have been developing a genetic labeling method, to follow *in vivo* the cellular mechanisms of ms-neuron branching during development (time-lapse imaging), as well as resolving the ultra-structure of axon branches using Correlative Light and Electron Microscopy (CLEM). We are now conducting a live-imaging analysis of single genetically labeled ms-neurons comparing growth cone dynamics in wild type and various mutant neurons, which complements and significantly improves our genetic analysis of axon branching.

379A The PP2A serine/threonine phosphatase complex functions in regulating dendritic diversification *Shatabdi Bhattacharjee*, Uy Nguyen, Daniel Cox. Neuroscience Institute, Georgia State University, Atlanta, GA.

The array of morphological diversity exhibited by neurons is critical in specifying patterns of synaptic connectivity. Elucidating the molecular mechanisms regulating dendritic diversification is therefore essential to understanding the formation and modulation of functional neural circuitry. While studies have demonstrated that transcription factors (TFs) function in specifying dendritic diversity, the molecular mechanisms via which this is achieved remain incompletely understood. A major target of TF regulation is the control of dendritic cytoskeletal architecture and dynamics. The TFs Cut and Knot are known to regulate dendritogenesis via cytoskeletal modulation. To systematically characterize the downstream effectors of these TFs, we conducted neurogenomic analyses of Cut and Knot transcriptional programs from which we identified over 200 putative transcriptional targets. We discovered that Cut positively regulates the expression of the PP2A complex, a serine/threonine phosphatase that is evolutionarily conserved from yeast to mammals. In *Drosophila*, the PP2A complex is composed

of a catalytic subunit encoded by *microtubule star* (*mts*), a scaffolding subunit encoded by *PP2A-29B* and one of four alternate regulatory subunits encoded by *widerborst* (*wdb*), *twins*, *PP2A-B'* and *CG4733*. Consistent with Cut regulation, *mts*, *PP2A-29B* and *wdb* exhibit very high levels of expression in dendritically complex CIII and CIV neurons relative to simpler CI neurons. Mutant and RNAi analyses of *mts* and *PP2A-29B* reveal severe reductions in CIV dendritic complexity and *wdb* appears to function as the regulatory subunit, as disruptions in the other three subunits had no morphological effect. In contrast, defects in *mts* and *PP2A-29B* in CI neurons leads to increased dendritic complexity via *de novo* filopodia formation, whereas *wdb* appears largely dispensable, suggesting a more specific role of Wdb in complex dendritic arborization. Suppressor analyses indicate that Cut acts via Mts and PP2A-29B to restrict F-actin rich filopodia and promote microtubule (MT)-based dendritic extension. Consistent with this, live imaging reveals that *mts* and *wdb* mutations lead to MT destabilization, whereas the effects of these genes on F-actin are distinct, with Mts restricting F-actin levels and Wdb promoting F-actin levels. Moreover, overexpression analyses reveal dendritic shape is sensitive to expression levels of different complex components, which likewise have distinct impacts on the cytoskeleton. Thus in contrast to previous findings that link Cut to regulation of the actin cytoskeleton, our findings implicate Cut in modulating both MT and F-actin cytoarchitecture via PP2A thereby molecularly promoting dendritic diversity.

380B Motor neuron-glial interactions require distinct contribution from BMP ligands, Dpp and Gbb *Mathieu BARTOLETTI*, Aaron HELD, Laura RAND, Kristi WHARTON. Brown University, Providence, RI.

The nervous system of vertebrates is a complex circuitry made of several cell types that interact with each other. In vertebrates, glia appear to have a major role in several processes during brain circuitry development, such as axonal growth, neuronal progenitor proliferation, neuronal differentiation, as well as during later life by guaranteeing neuronal maintenance and function. They accomplish these tasks through intimate interactions with neurons. Consequently, the communication between different types of glial cells with neurons is crucial for establishment and maintenance of a functional neural circuit. As in vertebrates, the Drosophila glia are a major component of the nervous system and with many fewer neurons and glial cells overall, Drosophila is a powerful system to study the communication between different glial subtypes and neurons. We have found that Bone Morphogenetic Protein (BMP) 5/6/7 and BMP2/4 ligand orthologues, Glass bottom boat (Gbb) and Decapentaplegic (Dpp), respectively, contribute in distinct ways to glial-neuron interactions. gbb and dpp are expressed in discrete sets of cells in the Drosophila brain with gbb predominantly expressed in glial cells. Using RNAi to knock down gene expression, we found that glial cell expression of gbb is necessary for a normal synapse growth at the neuromuscular junction (NMJ), suggesting that Gbb acts from the glia to regulate motor neuron development. Interestingly this reduced growth of the NMJ is accompanied by a strong reduction of the active form of the BMP effector, phosphorylated Mother against dpp (pMad). This suggests that glial cells and motor neurons communicate through a canonical Smad-mediated pathway to allow proper synapse development. The expression of dpp is not located in glial cells but rather in a subset of dorsal and ventral motor neurons. The distinct location of both BMP ligands and their involvement in glial-neuron interaction during development highlight the importance of the BMP pathway in neural circuitry. We will present our findings thus far as we continue to study the molecular and cellular mechanisms underlying the distinct roles of BMP ligands in neural development and circuitry.

381C Secreted Notum Coordinates Synaptogenesis via Extracellular Regulation of Wnt Wingless *Trans*-synaptic Signaling *Danielle Kopke*, Sofia Lima, Kendal Broadie. Biological Sciences, Vanderbilt University, Nashville, TN.

Drosophila Wingless (Wg) is the founding member of the Wnt intercellular signaling ligands, which play critical roles in an array of developmental processes. At neuronal synapses, Wnts act as potent regulators of connectivity, structural architecture, neurotransmission strength and activity-dependent (A-D) plasticity. At the *Drosophila* neuromuscular junction (NMJ) model glutamatergic synapse, Wg is secreted from neuronal presynaptic boutons and glia to bind pre- and postsynaptic Frizzled-2 (Fz2) receptors and activate downstream signal transduction. Recently, the Wg regulator Notum was characterized in detail as a secreted carboxylesterase (not a phospholipase as previously reported) that cleaves an essential lipid moiety from Wg (palmitoleate), rendering it unable to bind Fz2. As the first discovered extracellular deacylase, Notum is a highly conserved secreted feedback antagonist of Wnt signaling. Our goal has been to study Notum roles at the *Drosophila* NMJ synapse in the context of regulating *trans*-synaptic Wg signaling. Using immunohistochemistry (IHC), we find that Wg signaling at the synapse is upregulated in *notum* mutants, including both elevated Wg ligand levels and increased Frizzled Nuclear Import (FNI) signaling in the postsynaptic muscle. We show Notum secreted from the muscle co-localizes with Wg in the extracellular synaptomatrix surrounding developing boutons. Confocal imaging reveals *notum* mutants develop overgrown synaptic architecture. Functionally, *notum* mutants develop more synapses and stronger NMJ synaptic transmission measured using two-electrode voltage-clamp (TEVC) electrophysiology. Consistently, *notum* mutant larvae have a faster reaction time, revealed using coordinated roll-over behavior assays. Structural, functional and behavioral changes are phenocopied by Wg overexpression, showing that Notum acts as a negative regulator of Wg

signaling. At individual synaptic boutons, *notum* mutants have a synaptic vesicle (SV) cycling defect, revealed both by FM1-43 dye live imaging and transmission electron microscopy (TEM). Upon A-D depolarization, *notum* mutant boutons load less dye and contain enlarged vesicles, but exhibit an overall decrease in SV density. Importantly, genetically reducing Wg to wildtype levels in *notum* null mutants suppresses the synaptogenic defects, proving Notum is a negative regulator of Wg *trans*-synaptic signaling. Taken together, these wide-ranging studies reveal a novel extracellular enzymatic mechanism for the regulation of *trans*-synaptic signaling, once again highlighting the importance of the synaptomatrix in shaping the intercellular communication at the heart of synaptogenesis. This research has been entirely supported by NIH grant MH084989 to K.B.

382A Tao negatively regulates *Drosophila* neuromuscular junction formation *Ryan Salemme*, Stephen Politano, Toren Bakula, Pam Vanderzalm. John Carroll University, University Heights, OH.

The Hippo pathway is well-established as a negative regulator of growth in epithelial tissues, but it also regulates central nervous system development. We tested whether Hippo signaling might similarly regulate growth and development at the synapse, specifically at neuromuscular junctions (NMJs). We overexpressed or knocked down (by RNAi) Hippo pathway gene expression presynaptically, including *hippo, Merlin, Tao*, and the output of the pathway, *yorkie.* We found that only the serine/threonine kinase Tao, and not other members of the Hippo pathway, altered the number of boutons at an NMJ.

Tao is expressed in the nervous system, and overexpression or loss of *Tao* postsynaptically does not affect the gross morphology of NMJs. Loss of presynaptic Tao leads to overgrowth of NMJs and an increase in the number of active sites. Presynaptic overexpression of *Tao*, but not a kinase-dead version of *Tao*, lowers the number of boutons at the NMJ. These opposing phenotypes suggest that the development of the NMJ is sensitive to Tao kinase activity levels. However, since no other Hippo pathway genes tested resulted in a similar phenotype, we believe that Tao is exerting its effect on NMJ development through a Hippo pathway-independent mechanism, which we are currently investigating.

383B Orchestrating neurons that derive from different neuronal progenitor pools in the fly visual center *Makoto Sato*, Takumi Suzuki. Institute for Frontier Science Initiative, Kanazawa University, Kanazawa, Ishikawa, Japan.

During brain development, various types of neuronal populations are produced from different progenitor pools to produce neuronal diversity that is sufficient to establish functional neuronal circuits. In many cases, neurons that derive from different progenitor pools are mixed as a result of neuronal migration, and are correctly connected with their partners. This process is especially important to increase the diversity of neuronal circuit by utilizing limited number of neuronal progenitor pools. The following steps are important to orchestrate neurons that derive from different progenitor pools. First, the identity of different progenitor pools must be precisely specified. Second, the arrangement and migration patterns of neurons of different origins must be precisely regulated. Although detailed molecular mechanisms of these processes have been obscure in vertebrate brain systems, the medulla, the largest component of the *Drosophila* visual center, is an excellent model system to study this problem.

In the course of medulla development, different types of neurons are produced from two progenitor pools: the outer proliferation center (OPC) and glial precursor cells (GPC). Here, we show that Wnt/Wingless signaling controls the neuronal progenitor pool identity by specifying the fate of OPC. Additionally, we show that GPC-derived neurons migrate tangentially into the developing medulla cortex and are mixed with OPC-derived neurons. We also show that the tangential migration of these neurons is regulated by Slit-Robo signaling through interactions with adjacent cells, suggesting that conserved axon guidance signaling is involved in the interactions between neurons of different origins.

384C NF-Y acts in a temporal cascade in *Drosophila* medulla neuroblasts to control neural fates *Thinh N Tran*[1], Nikolaos Konstantinides[2], Claude Desplan[2,3]. 1) New York University Abu Dhabi, UAE; 2) Department of Biology, New York University, NY, USA; 3) Center for Genomics and Systems Biology, New York University Abu Dhabi, UAE.

Temporal patterning is used in both vertebrate and invertebrate nervous systems to generate neuronal diversity from neural stem cells. In the developing *Drosophila* optic lobe, a cascade of five temporal transcription factors (tTFs) – Homothorax (Hth)/Distalless (Dll), Eyeless (Ey), Sloppy-paired (Slp), Dichaete (D), and Tailless (Tll) – is sequentially expressed in the neuroblasts of the outer proliferation center (OPC) that generate the medulla neuropil of the optic lobes. Neuroblasts generate different neuronal types in each temporal window. The progression of the temporal series relies on the cross-regulation of the tTFs. Ey, Slp, and D activate the expression of the next tTF, while Slp, D, and Tll repress the previous one. The lack of cross-regulation between Hth/Dll and Ey suggests the existence of at least one missing tTF that acts between the two. We used modENCODE transcriptome data to identify transcription factors expressed in the *Drosophila* larval head. Through a screen with antibodies and RNAi to test the potential of ~190 transcription factors for being part of the temporal series, we identified Nuclear factor Y (NF-Y) as

our primary candidate. NF-Y is a CCAAT-binding heterotrimeric factor that has been shown to function in eye disc specification and photoreceptor development. Knockdown of NF-YB, one of NF-Y's three subunits, in the OPC leads to the expansion of Hth/Dll expression and the loss of Ey expression in neuroblasts. This result suggests that NF-Y acts between Hth/Dll and Ey in the temporal cascade to suppress the previous and activate the next tTF. This completes the optic lobe neuroblast temporal series and will allow us to further examine how temporal patterning develops, progresses, and contributes to the generation of neuronal diversity in the optic lobe. The identification of yet another tTF that plays an important role in optic lobe development demonstrates once more how gene regulatory networks, such as the one between Hth/Dll, NF-Y, and Ey, are redeployed in different systems during evolution.

385A The effect of developmental exposure to nicotine on dopamine expression in *Drosophila melanogaster* *Norma Velazquez Ulloa*[1], Melanie Morris[2], David Valenzuela[3]. 1) Biology Department, Lewis & Clark College, Portland, OR; 2) University of Washington, School of Medicine, WA; 3) Madison High School, Portland, OR.

Tobacco addiction is a complex issue that affects millions of individuals each year and kills up to half of all users. Nicotine is the primary compound in tobacco associated with its addictive properties. Even though the receptors for nicotine are known, the mechanisms that mediate nicotine addiction are not well understood. We have developed a *D. melanogaster* model for developmental nicotine exposure that recapitulates several of the known effects of nicotine. Now we are investigating whether changes in the development of the dopaminergic system could underlie some of the behavioral effects of developmental nicotine exposure. We hypothesize that nicotine exposure during development could affect neurotransmitter specification, and hence the number of neurons expressing a given neurotransmitter by adulthood. Chronic exposure to nicotine in other model organisms has been shown to increase expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. We tested whether developmental nicotine exposure in *D. melanogaster* changes the expression of dopamine by adulthood. Flies of the *white* Berlin strain were reared on control or nicotine food and eclosion rates were determined to make sure that the nicotine treatment had the expected effect. Once the flies eclosed, they were collected for immunostaining. An antibody to TH was used to mark dopaminergic neurons in adult brains and confocal images were taken. The staining brightness and number of TH+ neurons were determined. We found no significant difference in brightness nor in the number of neurons expressing TH in the PAM and PAL clusters in adult brains of flies reared on control or nicotine food. Next, we have started testing whether nicotine exposure during development affects dopaminergic expression in larvae brains. So far we have seen no differences in the DL1 and DL2 clusters in the control versus nicotine rearing condition, but there was an increase in the number of TH positive neurons in the DM cluster. These data are still preliminary and we are increasing our sample size. In addition, we will examine changes in TH expression by qPCR in adult brains and larvae brains after nicotine exposure.

386B Susceptibility *Lipid Phosphate Phosphatase gene* possibly involved in Glial morphogenesis. *Jin-Hyeon Choi*, Sae-E Song, Mi-hwa Lee, Sang-Hak Jeon. Seoul National University, Seoul, South Korea.

Glial cells have various functions such as supporting neuron nutrients, defending neuronal environments, and axon pathfinding. *Drosophila* glial cells are highly similar to human glial cells. Studying *Drosophila* glial cells is important to study human neurodegeneration. We figured out a novel *Drosophila* gene which encodes *lipid phosphate phosphatase(LPP) gene*. The function of *Drosophila LPP gene* is known as trachea development, germ cell migration, and septate junction. In this study, we discovered another function through novel *Drosophila* gene. Novel *LPP gene* is highly similar to repo pattern *in situ* data. Using the GAL4/UAS system, we suppressed the *LPP gene* in glia cells and eye. We found aggregation of glial and ommatidia cells, which suggests that LPP genes were possibly involved in glia morphogenesis.

387C Molecular Mechanisms Regulating Glial Development *Jennifer Jemc*, Diana Luong, Victoria Hans, Asmabanu Patel, Christina Frasik, Danielle Talbot, Roni Milgrom. Dept. of Biology, Loyola University Chicago, Chicago, IL.

Glial cells perform numerous functions to support neuron development and function, including axon wrapping, formation of the blood brain barrier, and enhancement of synaptic transmission. We have identified a novel gene, *raw*, which functions in glia of the central and peripheral nervous systems. Reducing *raw* levels in glia results in morphological defects in the brain and ventral nerve cord, as well as a reduction in the number of glia along peripheral nerves and in the larval eye imaginal disc. Examination of peripheral nerve structure by transmission electron microscopy upon *raw* knockdown reveals clustering of neuron axons, suggesting defects in neuron ensheathment. While peripheral glia are specified normally in the embryo, the reduced number of glia along peripheral nerves and in the eye imaginal disc suggests that glia fail to proliferate or undergo cell death when *raw* levels are reduced. We are examining levels of cell proliferation and cell death in the peripheral nerves and the developing eye-brain complex upon *raw* knockdown. Finally, we are interested in how impaired glial development affects neuronal development and function. Crawling assays reveal that larvae exhibit reduced locomotion

when *raw* levels are reduced in glia. In the context of the eye, *raw* knockdown results in defects in axon targeting to the optic lobe. Our data suggests that Raw plays a critical role in glia development, as well as functioning cell non-autonomously to regulate axon targeting and neuron function. Current studies are aimed at identifying the signaling pathways through which Raw functions to promote nervous system development and function.

388A Effects of Hippo signaling pathway genes on glia proliferation during *Drosophila* embryogenesis. Hyeon-Pyo Shim, *Yejin Park*, Ahyoung Kwon, Hyewon Seo, Sang-Hak Jeon. Seoul Nat'l Univ., Seoul, South Korea.

Glial cells have many roles during neurogenesis such as axon pathfinding, cell fate regulation. Hence, most of neuronal diseases are related with glia and their normal development at early embryonic stage is important. It is unclear that the mechanism of how many glial cells are produced by the neural glial blast. Expanded, a hippo signalling pathway component, activate downstream protein Hippo, and subsequently repress Yorkie(Yki). Yki is a transcription factor of genes whose role is proliferating cells. In this study, we figured out function of expanded focusing on glial cell proliferation. In expanded loss-of-function mutants, the number of longitudinal glial cell increased, and in gain-of-function mutants, it decreased. When hippo was mutated, longitudinal glial cell number partially increased. Using GAL4/UAS system, we overexpressed Yki and found that the number of glial cell increased. These results suggest that Expanded controls glial cell number through Hippo signaling pathway.

389B Assessment of Developmental Toxicity Potential of Glyphote-Based Herbicides Using *Drosophila melanogaster* Primary Embryonic Stem Cell Cultures *Jesse A Argueta*, Anel Torres. California State University, San Bernardino, San Bernardino, CA.

Glyphosate-based herbicides (GBH) are widely and most commonly used in the world of agriculture. A number of earlier developmental toxicity studies, utilizing a number of model organisms, have indicated that GBH exposure can result in neural defects and cranial malformations in the developing fetus. *Drosophila melanogaster* embryonic cell culture system has been established as a robust *in vitro* assay to identify developmental toxicants (teratogens). Previously, more than 100 chemicals had been assessed for their teratogenic potential and the assay has a very low number of false positives or false negatives. The assay utilizes gastrula-staged *Drosophila* embryos which are homogenized into single cells, diluted into an appropriate cell density with Schneider's Drosophila medium that has been supplemented with FCS, and plated in 35mm dishes. The embryonic stem cells over the course of 24 hours go on to differentiate into a number of different cell types, but predominantly give rise to neuronal clusters (mostly cholinergic neurons) and myotubes. In this study, *Drosophila melanogaster* embryos were collected, cultured, and exposed to GBH at concentrations of 1x10-5mM, 5x10-5mM, and 1x10-4mM. Concentrations above 1x10-5mM yielded a significant decrease in the numbers of terminally differentiated neuronal clusters and myotubes, and therefore identified GBH as a potential teratogen. Future studies will include identifying if GBH targets only cholinergic neurons and if GBH exposure results in the induction of hsp 70 (a major stress protein). It is hoped that this assay can be used, along with a battery of other *in vitro* assays, as a screen for the large number of insecticides and pesticides awaiting comprehensive testing of their teratogenic potential.

390C Role of JAK/STAT signaling in the Optic Lobe Development *T. Yasugi*, T. Suzuki, R. Takayama, M. Sato. Infiniti, Kanazawa University, Japan.

To generate the adequate number of neurons and glia, proliferation and differentiation of neural precursor cells must be tightly regulated. However, mechanisms that control the behavior of neural precursor cells are still poorly understood. We have tried to uncover mechanisms which regulate proliferation and differentiation of neural precursor cells by using the Drosophila optic lobe as a model. The adult optic lobe is composed of four neural ganglia: the lamina, medulla, lobula, and lobula plate. Recently, it has been shown that lamina glia and lamina wide-field (Lawf) neurons in the medulla share common neural precursor cells. During larval and early pupal stages, lamina glia and Lawf neurons are differentiated from the precursor cells called glial precursor cells (GPCs) in the posterior region of the optic lobe.

Here, we demonstrate the role of JAK/STAT signaling for proliferation and/or differentiation of GPCs. We found the activation of JAK/STAT signaling in the posterior region of the optic lobe. Overactivation or attenuation of JAK/STAT signaling in GPCs showed increase or decrease of lamina glia and Lawf neurons, respectively, suggesting that JAK/STAT signaling is essential for generation of these cell types. We would like to discuss how the number of differentiated neural and glial cells are regulated in the optic lobe.

391A Regulation of *Drosophila* Glutamate receptor subunit abundance by *optimus-prime*, a novel mRNA associated gene *Dina Beeler*, David Featherstone. Biological Sciences, University of Illinois at 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). 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. To identify possible regulators of Glutamate receptors a biochemical screen was done using GluRIIA mRNA as a probe. A novel, highly conserved, and previously unstudied protein was identified. We named this novel gene, CG12149, '*Optimus-prime*'. Optimus prime is the founding member of a novel protein family, most closely related to midasins and dyneins, and contains three ATPase domains, a von Willebrand factor A domain and a potential RNA binding domain. Recently, genome-wide human disease association studies have linked SNPs in the human opr homolog to bipolar disorder, autism spectrum disorders, migrane and other cognitive and behavioral disorders. Western blot analysis of OPr expression in *Drosophila* third instar larvae determined the presence of protein in both body wall muscle and the central nervous system. To further characterize this gene an *OPr* null mutant was generated using CRISPR genome editing. Additionally UAS-OPr flies were made for studying the effects of targeted overexpression of OPr in the muscle. Current results have shown that manipulation of OPr expression levels does not affect GluRIIA or GluRIIB mRNA level or the distribution of mRNPs. However, overexpression of OPr in body wall muscle leads to a loss of both GluRIIA and GluRIIB abundance at the NMJ. Analysis of synaptic morphology showed no change in the number of presynaptic active zones or boutons at the NMJ in OPr mutants. Future studies will continue to investigate the mechanism by which OPr regulates GluRIIA and GluRIIB in *Drosophila*.

392B Formin-mediated regulation of dendritic cytoskeletal architecture and behavior in a model of Charcot-Marie-Tooth sensory neuropathy *Jenna Harris*, Ravi Das, Daniel Cox. Neuroscience Institute, Georgia State University, Atlanta, GA.

Specialized neural morphologies are required for detection and transduction of sensory stimuli and emerge via complex growth mechanisms modulated by intrinsic and extrinsic signaling coupled with activity-dependent regulation. The cytoskeleton is a defining component of eukaryotic cells, including neurons, and constitutes the foundation of their inner architecture. Despite many significant advances, it is not yet clear how genetically encoded growth rules are dynamically expressed through the local molecular interactions of cytoskeletal components driving cell-type specific dendritic arborization. Using neurogenomic analyses, we have uncovered transcriptional regulatory programs that combinatorially converge on select target effector molecules to sculpt dendritic architecture. Among the identified targets, we demonstrate that Formin3 (Form3) functions cell-autonomously in class IV (CIV) dendritic arborization (da) nociceptive sensory neurons to stabilize distal higher order branching. Live confocal imaging of multi-fluor cytoskeletal reporters and IHC analyses reveal the *form3* mutants exhibit specific collapse of the dendritic microtubule (MT) cytoskeleton, the functional consequences of which include defective dendritic trafficking of mitochondria and satellite Golgi. Neurologically, mutations in *INF2* (the human ortholog of *form3)* have been demonstrated to be causative for dominant intermediate Charcot-Marie-Tooth (CMT) disease E and CMT has been linked to various defects in mitochondrial dynamics. CMT is a progressive neurological disorder, and developmental analyses of *form3* mutants reveal a progressive dendritic arbor collapse throughout larval development. Moreover, CMT sensory neuropathies lead to distal sensory loss resulting in a reduced ability to sense heat, cold, and pain. To mimic disease-causing mutations in the DID domain of *INF2* we generated transgenic flies expressing the INF2 FH1-FH2 domains. Furthermore, live confocal imaging and IHC analyses reveal that the introduction of *INF2-FH1-FH2* into a *form3* mutant background exhibits a rescue of dendritic MT stabilization. Behaviorally, disruption of *form3* function in CIV nociceptive neurons results in a severe impairment of nocifensive behavior in response to noxious heat, which can be rescued by expression of *INF2-FH1-FH2*, revealing shared primordial functions of Form3 and INF2 in regulating dendritic development and nociception, as well as providing novel mechanistic insights into the potential etiological bases of CMT sensory neuropathies.

393C N-terminal Gcm Confers Specific Degradation Signals That Regulate *Drosophila* Gliogenesis *X. Xiao*[1,2,3], Y. Zhai[1,2,3], H. Mao[1,2,3], C. Chang[4], B. Tan[4], C. Chien[5], M. Ho[1,2,3]. 1) Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine, No.150 Jimo Road, Shanghai 200120, China; 2) Key Laboratory of Arrhythmias of the Ministry of Education of China, Shanghai East Hospital, Tongji University School of Medicine, No. 150 Jimo Road, Shanghai 200120, China; 3) Department of Anatomy and Neurobiology, Tongji University School of Medicine, 1239 Siping Road, Shanghai, 200092, China; 4) Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Kwei-San, Tao-Yuan, Taiwan; 5) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

A fundamental issue during nervous system development is how individual cells are formed from the undefined precursors. Differentiated neurons and glia, two major cell types mediating neuronal function, are acquired from immature precursors via a series of explicit controls exerted by transcription factors such as proteins in the family of Glial Cells Missing (Gcm). In mammals, Gcm proteins are involved in placenta and parathyroid gland development, whereas in the invertebrate organism *Drosophila*, Gcm proteins act as fate determinants for glial cells, regulate neural stem cell (NSC) induction and conversion, and promote glial proliferation. In particular, Gcm protein levels are

carefully tuned for *Drosophila* gliogenesis and their stability is under precise control via the ubiquitin-proteasome system (UPS). Here we describe a versatile form of Gcm, which contains a N-terminus amino acid substitution Arginine to Leucine (R59L) associated with the mammalian disease hypoparathyroidism. Our results show that Gcm^{R59L} protein is less stable and exhibits a shorter half-life, suggesting that Gcm^{R59L} is a faster degrading form of Gcm. The instability inferred from R59L is altered in the presence of a proteasome inhibitor and due to hyperubiquitination, indicating a potential involvement of UPS. Interestingly, Gcm^{R59L} proteins exhibit an altered profile for intrinsic phosphorylation status, leading to abnormal degradation. Finally, *in-vivo* analysis shows that Gcm^{R59L} is less competent in inducing *Drosophila* gliogenesis, reinforcing the significance of protein stability in the contexts of transcription activation and disease-related mechanism.

394A Glia-derived ubiquitin E3 ligase dSmurf regulates glia-MB axon interactions and *Drosophila* behaviors via Fused *Changyan Chen*[5], Shuai Yin[1,2,3], Yijing He[1,2,3], Xuebing Chen[1,2,3], Haiwei Pi[4], Lei Xue[5], Margaret S. Ho[1,2,3]. 1) Research Center for Translational Medicine; 2) Key Laboratory of Arrhythmias of the Ministry of Education of China, Shanghai East Hospital, No. 150 Jimo Road; 3) Department of Anatomy and Neurobiology, 1239 Siping Road, Tongji University School of Medicine, Shanghai, 200092, China; 4) Department of Biomedical Sciences, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan; 5) Institute of Intervention Vessel, Shanghai 10th People's Hospital, Shanghai Key Laboratory of Signaling and Diseases Research, School of Life Science and Technology, Tongji University, 1239 Siping Road, Shanghai, 200092, China.

Glia-neuron interactions are essential integral parts within neural circuits that underlie higher order behavioral tasks such as learning and memory and motor control. Similar to their mammalian glial cell counterparts, *Drosophila* glia phagocytose and clear debris from synapses and degenerating axons, and secrete ligands to signal neuronal remodeling. Here we present evidence that the ubiquitin E3 ligase dSmurf, a factor crucial for developmental processes such as ovary and adult germline stem cells (GSCs) differentiation and wing disc development, functions in glia to regulate the formation of mushroom body (MB), the main control center for olfactory-associative learning and locomotor control. During metamorphosis, glial-specific knock down of dSmurf expression results in selective thickening of MB α and βlobes. In contrast, dSmurf overexpression in glia leads to severe disruption of MB α and β lobes, a defect last until the end of metamorphosis and persistent into adult stage. Strikingly, upon overexpression of Fused (Fu), a serine/threonine kinase involved in Hedgehog and BMP signaling pathways, the thinning of MB α and β lobes is restored, suggesting that glial dSmurf and Fu interact cooperatively to regulate MB formation in a non-cell-autonomous manner. Furthermore, glial-specific dSmurf expression causes short-term memory defects and adult climbing and paralysis deficits, suggesting its involvement in olfactory-associative learning and epileptic behavior. Taken together, our results indicate that dSmurf plays pivotal roles in glia-mediated MB formation and locomotor activity. In addition to g axon pruning, glia regulate formation of MB a and b lobes in a timeline later than g axon pruning, suggesting a sequential requirement for glia in MB neuronal remodeling, hence the corresponding function and activity. These findings reinforce the importance of glia in neuronal function and circuit-controlled behavior.

395B Basic and invertebrate neuroscience funding at NINDS *Daniel Miller*, Anna Taylor. NINDS, National Institutes of Health, Bethesda, MD.

NINDS fulfills its mission “to seek fundamental knowledge about the brain and nervous system and to use that knowledge to reduce the burden of neurological disease” by supporting projects that range from Phase III clinical trials to basic research in model organisms. As a critical component of this mission, NINDS is deeply committed to supporting basic neuroscience research not explicitly targeting a specific disease. In times of stagnating science funding and contracting NIH paylines, the research community may understandably fear that basic science is at a funding disadvantage. In 2014, NINDS released its analysis tracking the proportion of the NINDS budget spent on competing basic neuroscience vs disease-related research projects over time. It reveals a steady and disquieting decline in the proportion of the NINDS budget spent on basic research between 1997 and 2013. However, review of unfunded applications over this time frame revealed that the decline in basic research funding was correlated with a parallel decline in the number of basic science applications submitted for review – despite consistently higher success rates of basic science proposals compared to disease-related basic, translational, or clinical proposals. We have now expanded this analysis to include funding trends for research using invertebrate model organisms in the NINDS portfolio, as well as funding rates for *Drosophila* applications in the NIH BRAIN Initiative. Surprisingly, total NINDS research dollars spent supporting *Drosophila* grants has remained relatively constant since 2008, and success rates of *Drosophila* applications at NINDS are consistently 5-10% higher than overall rates. We include data on the role of *Drosophila* in the BRAIN initiative projects, and direct viewers to NINDS Funding opportunities to support basic research and research using simple model systems at NINDS

396C Serotonin Receptors Modulate Responses to Methamphetamine and Cocaine *Charles Nichols*, Melaine Sebastian, Jaime Becnel. Pharmacology, LSU Health Science Center, New Orleans, LA.

Despite the study of drug abuse and dependance for decades, there are no FDA approved therapies to treat abuse

and addiction of stimulant drugs like cocaine and methamphetamine. Although dopamine is a crucial neurotransmitter involved in the response to stimulant drugs, serotonin has been found to also be important in mammalian response both as a dopaminergic modulator and by direct action. Cocaine is a potent blocker of the serotonin transporter, where its action there mediates some of its effects, and there have been many studies exploring the role of individual serotonin receptors in its mechanism of action, with a focus on the 5-HT_2 and 5-HT_{1A}receptor families. With respect to amphetamines, serotonin also plays important roles in the elicited behaviors. 5-HT_2 receptors are known to modulate levels of dopamine and influence methamphetamine-induced behaviors, and 5-HT_{1A} receptors are also known to contribute to the behavioral effects. Although serotonin and 5-HT_2 and 5-HT_{1A} receptors have been shown to modulate stimulant responses, and they are highly expressed in several brain regions implicated in reward and dependence, the underlying molecular mechanisms of this modulation at the cellular and circuitry level related to the effects of cocaine and methamphetamine remain unknown. The fly represents a powerful genetic model organism to investigate circuitry and molecular interactions underlying drug abuse. Drosophila express dopamine D1 and D2-like receptors that are known to mediate the effects of stimulants, as well as serotonin 1, 2 and 7 receptors that each are involved in a wide range of behaviors. Despite conserved behavioral responses to CNS stimulants between fly and mammal, and the involvement of dopamine, little is know regarding the underlying neural circuitry. We have been utilizing DREADD receptor technology to begin mapping circuits and cells in the fly brain necessary for behavioral responses to cocaine and methamphetamine. Several of these are represented by GAL4 drivers for aspects of the serotonergic system. Further, we have employed pharmacological approaches to probe the contribution of individual serotonin receptors in responses to CNS stimulants, where each major receptor family appears to play a role.

397A Nitric oxide signaling and ethanol sensitivity in *Drosophila* *Rebecca Schmitt*, Mike Grotewiel. Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA.

Nitric oxide (NO) is a key gaseous neurotransmitter in both vertebrate and invertebrate nervous systems. Nitric oxide synthase (NOS) produces NO by way of conversion of L-arginine to L-citrulline. NO signals via soluble guanylate cyclase as well as other downstream molecules. Although a few studies implicate NO in ethanol sedation behavior in rodents, the underlying mechanism of NO-mediated changes in this ethanol-related behavior has not been identified. We are using flies to investigate this mechanism. Fly NOS is encoded by a single gene that is flanked by two unrelated genes, CG6700 and CR44581. In addition, the genes CG6508 and CG17134 are located within the Nos transcription unit. We have backcrossed and characterized three putative Nos mutants, each containing a single piggyBac transposon inserted within Nos. Two of the transposon insertions are near the 5' end of the Nos locus, while one is located near the 3' end. Flies homozygous for each of these transposon insertions had decreased levels of total Nos mRNA expression, indicating that the insertions cause partial loss of function in Nos. Homozygous or transheterozygous flies containing the partial loss of function Nos alleles had increased ethanol sedation sensitivity compared to isogenic controls. Nos mutant flies, however, did not have altered basal locomotion or internal ethanol levels, indicating that the changes in ethanol sedation sensitivity were unrelated to disrupted ethanol uptake/metabolism or global behavioral defects. Furthermore, recovery from ethanol sedation was normal in Nos loss of function flies, suggesting that NO signaling might selectively influence the progression of—but not recovery from—ethanol sedation. We recently found that expression of CG6508 and expression of CG17134 (genes nested within Nos) are altered in flies with the three transposon insertions in the locus, raising the possibility that these two genes might also be involved in ethanol sedation. We are currently investigating whether decreased function of NOS is causally related to increased ethanol sensitivity or, alternatively, if the phenotype is due to altered expression of one or both of the nested genes within Nos. Our data are consistent with a model in which NOS and therefore NO signaling are required for normal sensitivity to, but not recovery from, ethanol sedation.

398B Role of Eclosion Hormone in *Drosophila* Ecdysis *Valeria Silva Moeller*[1], Javier Álvarez[1], Rubén Herzog[1], Robert Scott[2], Benjamin White[2], John Ewer[1]. 1) Universidad de Valparaíso, Valparaíso, Chile; 2) Section on Neural Function, LMB, NIMH, National Institutes of Health, Bethesda, MD, USA.

Ecdysis is a critical behavior used by arthropods to shed their old cuticle at the end of each molt. This innate behavior is controlled through the sequential and complex action of several hormones and neuropeptides including ETH (ecdysis triggering hormone) and EH (Eclosion hormone). In this study we focus on the role of EH and its receptor, EHR, during pupal ecdysis. Through the use of immunocytochemistry in combination with EHR- and ETHR (ETH receptor)-GAL4 drivers, we found that EHR and ETHR share similar expression patterns in peptidergic neurons, and are co-expressed in neurons that produce EH, CCAP (crustacean cardioactive peptide), kinin, FMRFamides, MIPs (myoinhibitory peptides), and bursicon, most of which are known to participate in the control of pupal ecdysis. EHR is also expressed in a large number of neurons whose identity and function is currently unknown. In addition, EHR is expressed throughout the trachea including in the epitracheal endocrine cells that produce ETH (Inka cells). We are investigating the role of these different neurons and cells by determining the consequences on ecdysis of driving EHR RNAi in subsets of EH targets. We are also investigating their role by performing real-time imaging of intracellular calcium dynamics triggered in EHR neurons in response to *in vitro* stimulation with ETH.

These result will contribute to our understanding of the function of the EH neuropeptide during *D. melanogaster* ecdysis behavior.

399C Executive and Adaptive Components of a Motivated Behavior Are Differentially Governed by Distinct Isoforms of a Hormone Receptor *Feici Diao*, Rebecca vaadia, Benjamin White. national institute of mental health, Bethesda, MD.

In flies, as in mammals, survival and reproduction require relatively stereotyped behavioral programs that are often under the combined control of hormonal and environmental cues. In flies, expansion and hardening of the wings is an essential process that must be executed directly after metamorphosis and which requires activation of neurons that secrete the hormone bursicon. The bursicon-expressing neurons are subject to control from both inhibitory environmental signals and other hormones. One such hormone is Ecdysis Triggering Hormone (ETH), which is required for post-metamorphic emergence and is thought to coordinate this process with wing expansion, though little is known about the mechanism of ETH action. Using a novel technique to identify and manipulate neurons that express the two isoforms of the ETH receptor (i.e. ETHRA and ETHRB), we have shown that ETH acts not only directly on bursicon-expressing neurons, but also on the pathway that mediates the environmental inhibition of wing expansion.

By creating transgenic fly lines that express the yeast transcription factor Gal4 selectively in neurons that express either ETHRA or ETHRB, we have shown that ETHRA, but not ETHRB, is expressed in most bursicon-expressing neurons. Activation of the neurons that express both ETHRA and bursicon using UAS-TrpA1 rapidly induces wing expansion, even under adverse environmental conditions. Suppression of these neurons blocks wing expansion, indicating that this population of neurons is both necessary and sufficient to induce wing expansion. Interestingly, suppression of neuronal activity in all ETHR-expressing neurons except those that express bursicon, also rapidly induces wing expansion in confined environments. We trace this effect to suppression of neuronal activity in ETHRB-, and not ETHRA-expressing neurons. ETHRA-expressing neurons thus promote wing expansion, while ETHRB-expressing neurons appear to mediate its inhibition by environmental factors. Overall, our results suggest that ETH acts to positively and negatively regulate wing expansion by modulating both the neuroendocrine command of that process and its environmentally-mediated inhibition. The positive and negative control pathways are differentially governed by the two distinct ETHR isoforms.

400A The gut microbiota affects learning and immunity in *Drosophila* *Michael DeNieu*, Ryan Wagstaff, Mollie Manier. Biological Sciences, The George Washington University, Washington, DC.

Recent studies have shown that microbes inhabiting the gut have been implicated in the proper development and function of host metabolism, immunity, and behavior, and disruption of these microbial communities can lead to detrimental effects on host health. A complex set of reciprocal interactions among the microbiota, the immune system, and the central nervous system, known as the microbiota-gut-brain axis, regulates the microbiota and its effects on host function, yet little is known about the mechanisms by which communication occurs along the microbiota-gut-brain axis. To explore these mechanisms, we tested axenic (germ free) and conventional (normal microbiota) flies for their ability to learn and respond to immune challenge. To measure learning, we used an aversive phototaxis suppression assay in which a light source is paired with an aversive quinine stimulus in order to test the ability of flies to associate the positive light stimulus with the quinine and avoid moving towards it. To test for immune function, we inoculated flies with non-pathogenic *E. coli* and measured the response of key immune pathways through qPCR. Our results show that loss of the gut microbiota impairs learning and the ability of the immune system to properly respond to infection, and current work is investigating the extent to which loss of immune function interacts with the inability to learn.

401B Parallel Hormonal Pathways in *Drosophila* Courtship Memories *Sang Soo Lee*[1,2], Michael Adams[2,3]. 1) Neuroscience Graduate Program; 2) Dept. of Cell Biology and Neuroscience; 3) Dept. of Entomology, University of California, Riverside, CA 92521.

While it is commonly accepted that long-term memory (LTM) formation follows short-term memory (STM), recent studies have revealed parallel neural processes for each. Here, we provide evidence that hormonal state regulates STM and LTM through apparently distinct pathways in *Drosophila*. We used the courtship conditioning paradigm for memory, whereby courting males experiencing rejection by mated females subsequently court receptive virgin females less avidly. We report here that an ecdysis triggering hormone (ETH)-juvenile hormone (JH)-dopamine (DA) circuit axis is essential for STM recall in courtship-conditioned males during an early adult critical period and that loss of STM memory recall by suppression of ETH → JH signaling is rescuable by the JH analog methoprene. DA circuits involved in STM require expression of both JH receptors Met and gce. With regard to LTM, both ETH and JH signaling also are required, but unlike STM, both hormonal signals appear to have direct actions on target neurons. Increased ETH release from Inka cells enhances memory through *de novo* protein synthesis, whereas increased JH

release from but corpora allata does not, indicating that ETH directly affects the nervous system for LTM. Unlike STM, the JH → DA circuit is not required for LTM. We also found that both ETH receptor and JH receptor Met are required for LTM formation by performing conditional RNAi silencing in the CNS. Expression of ETH receptors in the male brain suggests that a subset of memory consolidation circuits may be regulated by ETH. Our study provides new insight into the role of hormonal state in social interaction-dependent behavioral plasticity.

402C Assessing motivational drive to attain ethanol in *Drosophila melanogaster.* *N. Mei*, R. Azanchi, K.R. Kaun. Neuroscience, Brown University, Providence, RI.

Understanding ethanol's complex effects on reward and motivation circuits in the brain is critical for the development of better biologically informed therapies for ethanol abuse and addiction. Recent advances in neurogenetics have highlighted *Drosophila melanogaste*r as an exciting model to study the effects of ethanol at the circuit and single neuron levels. However, methods for assessing motivation for drugs like ethanol are lacking in the *Drosophila* field. To address this methodological gap, we have developed new memory assays for investigating motivational drive for odors and/or vaporizable stimuli like ethanol. Preliminary results show that Drosophila can demonstrate both seeking and avoidance behaviors for ethanol. Further, high content analysis reveals a number of factors that affect the decisions to seek alcohol. Future studies will assess the necessity and sufficiency of specific neuronal circuits in ethanol mediated seeking and avoidance. These experimental paradigms for estimating motivational drive will allow for circuit, single neuron, molecular, and genetic analyses of ethanol's motivational effects. Our results will also help inform similar conserved circuit motifs in mammalian models.

403A Ras acts as a memory suppressor in the *Drosophila* mushroom body. *Nathaniel C Noyes*, Erica Walkinshaw, Ronald L Davis. Neuroscience, TSRI , Jupiter, FL.

The requirement for Ras/ERK signaling in mammalian memory formation is well established. Surprisingly, we discovered that Ras activity acts as a memory suppressor in *Drosophila*. Knocking down Ras in the mushroom body, a brain structure necessary for olfactory memory, enhanced memory without affecting acquisition. Memory was reduced in flies expressing an ERK pathway-specific constitutively active mutant of Ras but not in flies expressing an AKT pathway-specific version. Our deeper analysis, which focused on the types of memory formed after olfactory conditioning, showed that Ras knockdown produced an enhancement of the protein synthesis-independent and anesthesia-resistant memory forms of memory but decreased protein synthesis-dependent memory. Overall, these results indicate that Ras acts as a memory suppressor in *Drosophila* with effects on specific types of memory. Memory suppressor genes, like Ras, offer unique insights into the constraints that the brain has on memory formation.

404B Octopaminergic activity mediates state-dependent alcohol reward memory expression. *Kavin M. Nunez*[1], Reza Azanchi[2], Karla R. Kaun[2]. 1) Molecular Pharmacology and Physiology, Brown University, Providence, RI; 2) Neuroscience, Brown University, Providence, RI.

Alcohol is one of the most widely abused substances worldwide. Despite recent advances, the neural and molecular mechanisms through which alcohol affects the brain are poorly understood. Moreover, a changing environment further complicates factors by shifting the internal state (hunger, sadness, fear, etc.) of an animal. However, it is not well understood how this influences the neural and molecular mechanisms underlying alcohol reward. We sought to investigate the neural substrates necessary for state-dependent alcohol reward memory, specifically focusing on food-deprived states. Recent studies show that octopamine receptor expression, octopaminergic neuron activity, and neuronal morphology change upon food-deprivation. Thus, we used the UAS/GAL4 binary expression system to manipulate neurotransmission in octopaminergic neurons of fed and food-deprived flies during alcohol memory acquisition and expression. Thermogenetically blocking neurotransmission in octopamine neurons resulted in a loss of memory expression in food-deprived flies but not fed flies. Furthermore, tyramine-beta-hydroxylase mutants that lack the enzyme needed to synthesize octopamine, displayed a loss in memory expression in food-deprived animals. These results suggest that octopamine is necessary for alcohol reward memory expression in food-deprived states and may be encoding information regarding the internal state of an animal.

405C The nuclear receptor *Hr46/DH3* is required in the blood brain barrier for male courtship *Brigitte Dauwalder*, Chamala Lama. Dept Biol/Biochem, University of Houston, Houston, TX.

We have previously found that circulating factors in the hemolymph regulate courtship (1). How these factors negotiate the blood brain barrier (bbb) that separates hemolymph and courtship circuits is not known.

We have demonstrated that a change of sexual identity of bbb cells in mature males significantly compromises courtship without affecting the barrier function of the bbb (2). To identify sex-specific transcripts, we next performed an RNA analysis of isolated bbb cells and found a number of transcripts that are preferentially expressed in male cells. Among these are trancripts for the nuclear recpetor Hr46/DH3. Antibody staining confirmed the presence of

Hr46 in the subperineurial glia cells (SPG) of the bbb. Conditional reduction of Hr46 in adult male bbb leads to a significant reduction in male courtship without affecting the integrity of the bbb. These data suggest a physiological role of Hr46 in SPG cells and indicate that signaling processes play a role in the communication between hemolymph and courtship circuits.

(1) Lazareva et al. PLoS Genet. 2007 Jan 26;3(1):e16.
(2) Hoxha et al PLoS Genet. 2013;9(1):e1003217. doi: 10.1371/journal.pgen.1003217.

406A Characterization of a novel gene required for *Drosophila melanogaster* mating *Sadaf Zaki*[1], Peter Luu[1,3], Jaspal Sandhu[1], David Tran[1,2], Rachael French[1]. 1) Biological Sciences, San Jose State University, San Jose, CA; 2) Counsyl, Inc., South San Francisco, CA; 3) Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

Male *Drosophila* carry out a complex courtship ritual involving a specific sequence of steps, which depend on the processing of sensory cues by the central nervous system. The master regulatory gene *fruitless* (*fru*) controls behavioral sex determination in flies; the male-specific splice form, fru^M, is both necessary and sufficient for all aspects of the courtship ritual.

We have identified a gene, *Trapped in endoderm 1* (*Tre1*), that is essential for normal courtship behavior in male flies. *Tre1* encodes an orphan G-protein-coupled receptor that is required for both germ cell migration and establishment of cell polarity, but has not previously been implicated in courtship. The goal of this research is to characterize the role of *Tre1* in *Drosophila* mating behavior. We found that male flies in which *Tre1*-expressing cells are feminized through expression of the female-specific isoform of the splicing factor *Transformer (TraF)* display unusually rapid courtship initiation, and that this phenotype is recapitulated in flies carrying a loss-of-function mutation in *Tre1* (*Tre1EP496*). Our results suggest that a subset of male-specific neurons in flies function to reduce the speed of courtship initiation. To identify these *Tre1*-expressing cells, we employed confocal imaging in flies expressing GFP under the control of a *Tre1* enhancer trap (*Tre1-GAL4*). We found that *Tre1-GAL4* is expressed in the olfactory organs and olfactory processing centers of the male nervous system, with little to no expression in these regions in females. In addition, we found that this expression pattern was lost in male brains in which the Tre1 neurons are feminized via expression of *TraF*.

To confirm that the requirement for *Tre1* in courtship is specific to neurons, we drove expression of a double-stranded RNA interference construct targeting *Tre1* (*Tre1-RNAi)* using the pan-neuronal driver *elav-GAL4* and found that silencing *Tre1* in neurons leads to rapid courtship initiation. These data collectively indicate that *Tre1* is required to establish normal mating behavior, and that the *Tre1* neurons are required to slow down the courtship ritual, as well as suggest a role for *Tre1* in the processing and integration of sensory signals during courtship. Given its role in germ cell migration and establishment of neuronal polarity, we hypothesize that the signal that the Tre1 GPCR receives is involved in sex-specific neuronal pathfinding, and that disruption of this pathfinding leads to improper neuronal connectivity, culminating in altered courtship behavior.

407B Dystonia-gene homologues regulate sleep robustness and synaptic development in *Drosophila* K-F. Chen, *J. Jepson*. Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom.

Dystonia is the third most common neurological movement disorder, and is characterised by involuntary muscle contractions resulting in repetitive twisting movements or abnormal postures. In a subset of patients, these symptoms can be treated with L-DOPA, linking reductions in dopamine release to dystonia pathology. However, the majority of dystonia patients are unresponsive to L-DOPA. Furthermore, while several genes have been linked to L-DOPA unresponsive dystonia, in the majority of cases their neuronal functions are poorly understood, as is their link, if any, to dopaminergic signalling. *Drosophila* sleep-wake behaviour is strongly regulated by dopamine (Kume et al. (2005) *J Neurosci*). Interestingly, sleep loss was recently found in *insomniac* (*inc*) mutants (Stavropolous and Young (2011) *Neuron*; Pfeiffenberger and Allada (2012) *PLoS Genet*), and *inc* is the *Drosophil*a homologue of the dystonia gene *KCTD17*. Using a guilt-by-association strategy combined with transgenic RNAi, we identified a novel role for an additional dystonia-gene homologue, *neurocalcin* (*nca*), in promoting night sleep in *Drosophila*. *nca* is highly homologous to the mammalian neuronal calcium sensor *Hippocalcin* (*HPCA*), mutations in which cause Dyt2 torsion dystonia. Surprisingly, we found that sleep loss in *nca* knock-down flies was photoperiod (i.e day length) dependent, such that night time sleep loss was enhanced in short photoperiods and suppressed in long photoperiod conditions. Sleep loss due to *nca* knockdown requires the Dop1R1 dopamine receptor and the visual system, but not the circadian clock. We are also examining the roles of NCA and INC at the larval neuromuscular junction (NMJ), and our preliminary data suggests that structural synaptic plasticity and/or neurodevelopment is altered in mutants of dystonia-gene homologues. In summary, our work places a dystonia-gene homologue in a common pathway with the Dop1R1 dopamine receptor, supporting a link between altered dopaminergic signalling and dystonia pathogenesis. Our results further suggest that sleep levels in *Drosophila* are actively buffered against photoperiodic changes by dystonia-gene homologues; that this robustness-promoting role occurs independently of the circadian clock; and that

alterations in synaptic plasticity may represent an underlying cellular mechanism linking INC and NCA to sleep in *Drosophila*.

408C Regulation of sleep by the IGF-II mRNA-binding protein (Imp) in *Drosophila*
Zhenxing Liu, Yong Zhang. Biology, University of Nevada, Reno, NV.

Sleep is an essential behavior found in most species of the animal kingdom. However, the underlying mechanisms of sleep regulation remain largely unknown. The fruit fly, *Drosophila melanogaster*, has proven to be a valuable model for studying the regulation of sleep by circadian clock and homeostatic mechanisms. RNA binding proteins are crucial post-transcriptional regulators, which control all aspects of RNA metabolism. Here we identified that an RNA binding protein-Imp (IGF-II mRNA-binding protein) regulates sleep in *Drosophila melanogaster*. *Imp* deficient flies exhibit increases of day time sleep and increases of sleep latency as well as significantly decreasing night sleep. We further tested in which neurons Imp is required to regulate sleep by using multiple GAL4 drivers expressed in known sleep circuitry. Imp is expressed in a large population of neurons in the fly brain, including mushroom bodies and circadian neurons. Downregulation of *imp* in circadian neurons recapitulated the sleep phenotype observed in *imp* mutants. Remarkably, re-expression of Imp in circadian neurons rescued *imp* mutants sleep defects. Mechanical sleep deprivation in the imp downregulation flies showed normal sleep rebound, which indicates that Imp is not involved in sleep homeostasis. Taken together our results demonstrate that Imp is required in circadian neurons for sleep regulation.

Keywords: *Drosophila melanogaster*, *imp*, sleep

409A Excessive food intake after starvation increases sleep in *Drosophila melanogaster*
Josue Regalado, Jared Link, Yong Zhang. University of Nevada, Reno. 1664 N Virginia St. Reno, Nevada. 89557.

Sleep and feeding are two highly conserved behaviors necessary for survival. Both are tightly interconnected as people with sleep disorders have a higher propensity of developing disorders involving feeding or metabolism, and vice versa. Starvation can potently suppress the amount of sleep in mammals and flies (MacFadyen et al 1973; Keene et al 2010). In addition, postprandial satiety has been shown to promote a series of behaviors, such as termination of meals and sleep (Antin et al 1975; Gibbs et al 1973). However, the genetic and neural mechanisms that modulate sleep changes after satiety remain unclear. We use the fruit fly, *Drosophila melanogaster* as a model organism to address the interactions between sleep and feeding. We first starved wild type flies for 24 hours to induce high food intake, and monitored their sleep. We found that sleep is increased in the first 4 hours after starved flies were given food. To address the possibility that sleep increase was due to homeostatic sleep recovery from sleep lost during starvation, we tested *translin* mutant flies, which fail to display starvation-induced sleep suppression. *translin* mutants continued to show sleep increase after starvation, suggesting excessive food intake underlies sleep increase. Video analysis revealed a high proportion of flies feeding in the first 15-30 minutes after starvation that levels out after 30 minutes. In addition, quantification of food intake reveals that starved flies ingest significantly less food after 30 minutes of feeding. Together, these results suggest that 30 minutes of feeding after starvation is sufficient for flies to reach satiation. Future experiments will tighten the link between satiation and sleep increase by blocking food intake in starved flies and monitoring sleep.

410B Identification of Neural Circuitry and Genes that Control Sleep Need
L. Kendall Satterfield[1,2], Nicole Hoffner[1,3], Glen Seidner[1], James E. Robinson[1,2], Meilin Wu[1], Kurtresha Worden[4], Pavel Masek[5], Alex C. Keene[6], William J. Joiner[1,2,3]. 1) Department of Pharmacology, University of California, San Diego, La Jolla, CA; 2) Biomedical Sciences Graduate Program,, University of California, San Diego, La Jolla, CA; 3) Neurosciences Graduate Program, University of California, San Diego, La Jolla, CA; 4) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 5) Department of Biology, SUNY Binghamton, Binghamton, NY; 6) Department of Biological Sciences, Florida Atlantic University, Jupiter, FL.

The sleep/wake cycle is thought to be controlled by two main processes: a circadian clock that primarily regulates timing of behavioral arousal and a homeostatic mechanism that regulates duration of arousal according to sleep need. Although molecular and anatomical mechanisms underlying circadian clock function have been studied in detail, the homeostatic mechanism by which sleep need is sensed and discharged remains unknown. This process has been difficult to study because of genetic and financial limitations in mammals and methodological limitations in other model systems. Here we describe a high throughput assay to study neural circuitry and genetic underpinnings of sleep need in Drosophila. Using this assay we found that nighttime activation of three independent neurotransmitter systems promoted wakefulness. However, only sleep deprivation resulting from activation of cholinergic neurons was sufficient to elicit subsequent homeostatic recovery sleep. We mapped the subset of responsible neurons and found that they innervate the central brain and motor control regions of the thoracic ganglion. Blocking activity of these neurons suppressed recovery sleep but did not alter baseline sleep, further differentiating between neural control of sleep homeostasis and daily fluctuations in the sleep/wake cycle.

Importantly, selective activation of other wake-promoting neurons without engaging the sleep homeostat impaired subsequent short-term memory, thus providing evidence that neural circuits that regulate sleep homeostasis are important for behavioral plasticity. Together, our data suggest a neural circuit model involving distinct populations of wake-promoting neurons, some of which are involved in homeostatic control of sleep and cognition. Using our novel high throughput assay, we also report the results of the first forward genetic screen for mutants exhibiting impaired sleep homeostasis.

411C A novel screen identifies a role for the histone variant H3.3 and its chaperone Hira in *Drosophila* aggressive behavior *Shaun Davis*, Amanda Thomas, Lingzhi Liu, Ian Campbell, Herman Dierick. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Aggressive behavior is widespread throughout the animal kingdom as a means to compete for territory, food, and mates. However, a potential consequence from these aggressive encounters is the risk of physical damage and even death. While aggression is part of the normal repertoire of complex behaviors for many animals, excessive aggression also occurs in many human neurological and psychiatric disorders. The molecular and neuronal mechanisms underlying this trait in health and disease remain largely unknown. The identification of novel genes that regulate aggression through a forward genetic screen has so far never been attempted in any organism because the complex nature of the behavior makes screening prohibitively time consuming. We circumvented this challenge when we discovered a positive correlation between aggression and physical wing damage in male flies that were group-housed. Furthermore, this wing damage leads to a decrease in flight ability and an increase in latency to mate, suggesting that the damage is a negative consequence of aggressive behavior. Using this easy-to-screen wing damage phenotype we performed the first chemical mutagenesis screen to isolate mutants with increased aggressive behavior. After screening ~1,400 EMS-induced X-chromosome mutants for increased wing damage, we found 5 lines that also had increased aggression. Using whole-genome sequencing, meiotic mapping, and genomic duplication rescues, we identified the causal mutation in 2 of these strains. One of these affects the histone chaperone, Hira, which incorporates the variant H3.3 into actively transcribed chromatin. Loss of H3.3 recapitulates the aggression phenotype of *Hira* mutants, suggesting that the regulation of gene expression by chromatin states profoundly affects the organisms' behavior. In terms of human health, there are approximately 100 Mendelian disease genes that are associated with aggression in patients. Consistent with our results in flies, string analysis of these genes forms a tight network of proteins that is significantly enriched in chromatin modifying enzymes that interact with the HIRA chaperoning complex. Our aggression-induced wing damage screening approach in flies is useful to identify novel genes regulating this complex behavior and may also provide insight into human aggression.

412A Geotaxis Analysis of Nora virus Infected *Drosophila melanogaster* *Amanda McCown*, Abigail Benz, Devyn Crisman, Kimberly Carlson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a member of the picornavirus family that infects *Drosophila melanogaster* with no known pathogenic effects. One possible pathogenic effect of Nora virus that has not been studied is locomotor ability. In this study, geotaxis assays and longevity curves were used to determine if Nora virus infection has an effect on *D. melanogaster's* locomotor ability. Ten small cages were established each containing 60 virgin female flies. The cages were marked with a line two thirds from the bottom of the cage. Every third day since cage establishment, the flies were tapped to the bottom and given one minute to reach the top. The number of flies crossing the threshold line were recorded as were the dead flies. Longevity curves were created and examined using Student's paired t-tests with $p < 0.05$. The data demonstrated a significant decrease in geotaxis when the *D. melanogaster* were infected with Nora virus. The data demonstrate that geotaxis and locomotor dysfunction may be a pathogenic hallmark of Nora virus infection. Overall, a better understanding of Nora virus may give us insight into other viruses in the picornavirus family. 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.

413B Octopamine drives endurance exercise adaptations in *Drosophila* *Alyson Sujkowski*, Robert Wessells. Physiology, Wayne State University, Detroit, MI.

Endurance exercise is an effective therapeutic intervention with substantial pro-healthspan effects. 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 respond to an exercise stimulus, but do not experience the adaptive training response seen in males. Here, we demonstrate that sexually dimorphic exercise response is mediated by differences in neuronal activity. The activity of octopaminergic neurons is specifically required to induce conserved cellular and physiological changes seen following endurance training. We further demonstrate, for the first time, that increased excitability of octopaminergic neurons is able to confer a suite of pro-healthspan benefits even in sedentary *Drosophila*. These experiments suggest that differences in neuronal "fight-or-

flight" response during training mediate individual differences in exercise performance, and open the door for the development of potential therapeutics.

414C Novel olfactory coding mechanisms in response to DEET and other compounds *Jonathan Clark*, Jadrian Ejercito, Ryan Arvidson, Anandasankar Ray. Entomology, University of California, Riverside, Riverside, CA.

Volatile odor-based repellents offer a powerful approach for preventing the spread of insect-borne diseases such as malaria, Zika, and dengue. Despite extensive research efforts, the molecular and cellular mechanisms through which repellents such as DEET elicit aversion in insects are poorly understood and controversial. We have used sensitive electrophysiological and imaging analyses to study the responses to several repellents across the *Drosophila* antenna. Alongside, we tested novel odor-delivery methods that better replicate stimuli that cause aversion and found an unusual phenomenon not reported earlier: DEET and other repellents cause widespread effects across numerous chemosensory neurons in *Drosophila melanogaster*. Some repellents cause broad activation in OR and IR expressing neurons, but did not activate *Gr63a/Gr21a+* CO_2-sensing neurons, indicating that the mechanism is not generalizable to neural physiology but specific to certain ORN types. Others are characterized by a short burst of activity followed by a prolonged silencing of neural activity. Imaging of internal calcium levels in HEK293 cells indicate that at least one repellent causes internal calcium mobilization without lysing the cells, indicating that this repellent is generalizable to even mammalian cells. Pretreating the cells with the SERCA blocker thapsigargin abolished the calcium response to this repellent. Interestingly, some of the tested odors are attractants at lower concentrations, and the concentration at which the physiological effects occur correlate with behavioral valence reversal. Further experimentation with structural derivatives allowed us to identify physicochemical factors that correlate with the inhibition of neurons. The inhibition can be blocked in a dose-dependent manner by the addition of compounds with the opposite physicochemical factors. For one repellent class, the effects are consistent across multiple chemosensory neuron types, including ORNs and the GR-expressing CO_2 neuron, suggesting a common physiological mechanism that is likely conserved in other Dipterans such as mosquitoes. Our results uncover a novel mechanism for aversion to common, naturally occurring compounds, and a new potential avenue for repellent discovery.

415A Odorant sensitivity is regulated by Orco phosphorylation in Drosophila *Hao Guo*, Kishor Kunwar, Dean Smith. Departments of Neuroscience and Pharmacology University of Texas Southwestern Medical Center 5323 Harry Hines Blvd. Dallas, TX 75390-9111.

Modulation of sensory sensitivity allows useful information to be extracted from a broad range of fluctuating stimuli in the environment. The mechanisms underlying changes in sensitivity in insect olfactory neurons are poorly understood. We identified and knocked down 10 candidate kinases expressed in the Drosophila antenna and one, *PKC98E*, had a significant effect on odorant sensitivity. Classical and conditional mutants confirmed the phenotype. PKC98E localized to the cilia of olfactory neurons, the site of odorant transduction. We explored a likely target for phosphorylation, the common receptor subunit, Orco. We identified a single residue, S298 that impacts odorant sensitivity. $Orco^{S298A}$ mutants show reduced sensitivity to odorants and other, while the phosphomimetic mutants, $Orco^{S298D}$ have enhanced sensitivity to odorants compared to wild type controls. We generated phospho-specific antiserum to this position and discovered that odorant exposure reduces phosphorylation at S289 without affecting Orco localization. Moreover, in the Drosophila lipid flippase mutant, *dATP8B*, that has a poorly understood reduced odorant sensitivity phenotype, we found PKC98E is mislocalized, indicating that lipid translocation is necessary for the localization of PKC98E to the cilia. The level of phosphorylated Orco is also significantly reduced in *dATP8B* mutant. These findings reveal odorant exposure regulates Orco sensitivity through phosphorylation *in vivo*.

416B A broad-spectrum olfactory neuron for odorant valence determination through statistical modeling *Joel Kowalewski*[1], Anandasankar Ray[1,2]. 1) Interdepartmental Neuroscience Program, UC Riverside; 2) Department of Entomology, UC Riverside.

The ability to predict behavioral valance of chemicals from sensory neuron activity has been extremely challenging for the olfactory system in any organisms. Spiking activity from olfactory neurons informs aversive and attractive behaviors in *Drosophila melanogaster*. While prior research has shown odor preference of the simple *Drosophila* larvae to diverse odorants may be predicted with activity from as few as 5 Odorant Receptors (Ors), it is unclear which receptors are involved in the adult stage and to what extent Gr expressing neurons, such as ab1C, might contribute to behavior. Recently we have found that the CO_2-sensitive ab1C neuron expressing *Gr21a* and *Gr63a* responds broadly to a number of odorants via activation or inhibition. To that end, we compared predictions of the preference index (PI) of adult flies to 55 odorants tested in a T-maze using published activities of 24 Or-only model and one including ab1C neuron that also responds to many of these odorants. Regressing PI on the 24 ORs could explain 59% of the variation in behavior. After adding ab1C, however, the model improved significantly from 59% to 67. We next identified the minimum model that could maximize the prediction rate

while controlling for overfitting. Candidate models were initially assessed on multiple criteria, followed up by stepwise removal of predictors until reaching an optimal model. Cross validation was then applied to test performance. Because certain odorants could have biased the model selection, the best model, which included four Ors and ab1C, selection was performed iteratively using 2000 bootstrap samples. ab1C was most consistent, followed by the same 4 Ors. In fact, ab1C responses alone showed a significant ($p < 0.00005$) correlation to the behavioral PI and improved predictions with an Or-only model in our regression analyses. Interestingly, the Or-only model was non-significant ($p > .05$). These data suggest that ab1C activity accounts for a significant amount of variability in odor preference of flies in a T-maze.

417C Olfactory Preferences and Parasite-Host Interactions of the Mushroom Fly *Drosophila falleni* and its Parasitic Nematode *Howardula aoronymphium* *James Cevallos*, Ryo Okubo, Elissa Hallem. Microbiology, Immunology, and Molecular Genetics, University of California Los Angeles, Los Angeles, CA.

Several species of parasitic nematodes use *Drosophila* as a vector. Parasitic nematode infections lead to a number of devastating diseases and cause billions of dollars in economic loss each year. Although many investigations have studied the immune response to parasitic nematode infection, little is known about parasitic nematode ecology. In particular, little is known about how parasitic nematodes and their hosts respond to olfactory cues in their environment, how the olfactory preferences of parasitic nematodes differ from their hosts, and the extent to which parasitic nematode infection alters specific host sensory behaviors.

We are using the mushroom specialist *Drosophila falleni* and its parasitic nematode *Howardula aoronymphium* as a model system to address how the olfactory preferences of parasitic animals differ from those of their hosts and to test the hypothesis that parasites alter the olfactory preferences of their hosts. *D. falleni* and *H. aoronymphium* is an excellent model system because the olfactory preferences of uninfected and infected *D. falleni* can be ascertained through chemotaxis assays and parasitic infection is not lethal.

We examined the olfactory preferences in *D. falleni* and *H. aoronymphium* in response to a panel of eleven mushroom-derived odorants as well as six fresh or aged complex odors. We also examined the olfactory preferences of *D. melanogaster* and *C. elegans* in response to the same panel of odorants and complex odors to investigate similarities and differences in olfactory preferences between specialist and generalist organisms. Olfactory behavior was quantified using a chemotaxis index.

We found that *D. falleni* and *H. aoronymphium* possess unique olfactory preferences. We also found that specialist organisms, such as *D. falleni* and *H. aoronymphium,* share a narrower range of olfactory preferences than their generalist organisms, *D. melanogaster* and *C. elegans*. We also found that parasitic nematode infection does not alter host locomotion but does cause specific changes in host olfactory behaviors. In all, our investigation is shaping the understanding of parasite-host interactions and the evolution of olfaction over time.

418A The Cellular Basis of Behavioral Responses to Odor-Mixtures in *Drosophila* larvae Sylvia Karpio, Marian Chytry, Olivia Barker, Joseph Lis, Karolina Kir, Patrycja Matel, Jeewanjot Grewal, Kathryn Swain, *Scott A. Kreher*. Dominican University, River Forest, IL.

Most previous research in olfaction has been conducted on the genetic and cellular basis of detection of single-molecule odors; this was an essential beginning point, and has yielded our partial understanding of the sense of smell. However, most odors are actually mixtures of molecules. There are important and outstanding questions regarding how mixtures of odors are perceived. For example, if we know the behavioral responses to two odors individually, odor A and odor B, then how do animals behaviorally respond to a mixture of A and B? Is the effect additive, does one odor trump the other, or is the response to the mixture unpredictable from responses to the individual odors?

We have begun characterizing the behavioral responses of *Drosophila* larvae to mixtures of attractant and repellent odors. We have developed multiple behavioral arena configurations to assess repellent odors, and have found some putative repellent odors. Behavioral responses to repellent odors are dose-dependent and are eliminated upon mutation of the *Orco* co-receptor. The putative repellent odors have natural sources that may explain their negative valence.

Mixtures of repellent odors and known attractant odors elicit behavioral responses that resemble responses to the repellent alone; this effect is independent of many parameters of the behavioral assay, including attractant odor concentration and odor delivery method. Some wild type lines show variation in the odor-mixture behavioral response, which is evidence that this is not merely due to the physicochemical properties of the molecules alone. The putative repellent odors are detected by a non-overlapping subset of odor receptors and sensory neurons compared to attractant odors; mutations in the relevant odor receptors yield expected behavioral results.

We are extending this work by analyzing behavioral responses to an expanded set of odor-mixtures to understand how mild attractant odors or odors with neutral valence affect responses to strong attractants.

419B Organization and Function of *Drosophila* Odorant Binding Proteins *Jennifer Sun*[1], Nikki Larter[1,2], John Carlson[1,2]. 1) Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT; 2) Interdepartmental Neuroscience Program, Yale University, New Haven, CT.

Odorant binding proteins (Obps) are remarkable in their number, diversity, and abundance, yet their role in olfactory coding remains unclear. They are widely believed to be required for transporting hydrophobic odorants through an aqueous lymph to odorant receptors. We construct a map of the *Drosophila* antenna, in which the abundant Obps are mapped to olfactory sensilla with defined functions. The results lay a foundation for an incisive analysis of Obp function. The map identifies a sensillum type that contains a single abundant *Obp*, *Obp28a.* Surprisingly, deletion of the sole abundant *Obp* in these sensilla does not reduce the magnitude of their olfactory responses. The results suggest that this Obp is not required for odorant transport and that this sensillum does not require an abundant Obp. The results further suggest a novel role for this Obp in buffering changes in the odor environment, perhaps providing a molecular form of gain control.

420C Dissection of Bitter sensing Gustatory Receptors in *Drosophila melanogaster* *Seeta Poudel*, Yunjung Kim, Youngseok Lee. Biofermentation and Biofusion Technology, Kookmin University, Seoul, South Korea.

Insect gustatory receptors (GRs) are crucial for the taste study. The GRs impart insects to ingest the nutritive food while avoiding the toxic one; the former is controlled via the sweet-sensing GRs and the later via the bitter-sensing GRs. The diversity of the bitter compounds causes variation in response from bitter (GRs) in the gustatory receptor neurons (GRNs). In our present work, we studied 18 *Gr*-mutants for 11 different bitter compounds. The 11 of 18 *Gr*-mutants used in our study were confirmed as null mutants by RT-PCR and were tested for both behavioral and electrophysiological assay. First, we found that 3 GRs were required for both umbelliferone and coumarin avoidance behavior. Furthermore, we disentangled molecular knots via a novel molecular participant having function in detection of chloroquine, an anti-malarial drug. In addition, our electrophysiological recording data suggest that chloroquine-mediated activation of GRNs occur through S-type sensilla. We revealed that chloroquine causes inhibition of sugar sensing neuron in dose dependent manners. Intriguingly, it also causes flies to deprive in the sleeping pattern irrespective of the gender. Our study has unsnarled various molecular participants to sense tastes and deorphanized a naive receptor with a previously unknown bitter compound.

421A Modeling alcoholism in the fly: Circuit mapping of sexually-dimorphic alcohol behaviors *Annie Park*, Nigel Atkinson. University of Texas in Austin, Austin, TX.

Alcoholism is thought to be a product of alcohol-induced alcohol neural adaptions that gradually lead to uncontrollable and pathological patterns of ethanol consumption. Alcoholism research in flies has made important contributions to our understanding of how such adaptations occur. Flies are an ideal model system for this work because flies show most of the same alcohol responses as humans do. Like humans, in flies low doses of ethanol are excitatory while higher doses act as depressants and eventually produce sedation. Flies also acquire functional alcohol tolerance (alcohol-induced alcohol resistance) and show signs of alcohol withdrawal and a form of alcohol dependence. Furthermore the interest of the fly in alcohol can be modified by prior experience. Male flies normally find ethanol aversive and will not drink it. However, after exposure to ethanol vapor they acquire a preference for liquid food laced with ethanol. Females, on the other hand, show a different response to ethanol food. While an ethanol-naive male finds ethanol aversive even at low concentrations (~5%), the female is strongly attracted to ethanol laden foods even at relatively high concentrations (~10% ABV). Although appetitive and aversive behaviors have been mapped onto brain regions and circuits, the circuits modulating ethanol preference have yet to be identified. By transgenically feminizing brain regions and specific neural circuits we are mapping the origins of these sexually-dimorphic responses of flies to ethanol. In this report we also describe a new method for measuring ethanol consumption in individual flies. This method is used in conjunction with a camera-based positional tracking system that reports when the flies access the ethanol. This two-pronged approach provides for a robust analysis of the drinking patterns of male, female, and feminized male flies. In this manner we are identifying the neural circuits that underlie sexually dimorphic ethanol preference.

422B Neural Modulation of *Drosophila* Oviposition Circuit *Daniel J. Suto*[1], Sonali A. Deshpande[1], James D. Asuncion[1,2], David E. Krantz[1]. 1) Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, Los Angeles, CA; 2) Medical Scientist Training Program at the University of California, Los Angeles, Los Angeles, CA.

Biogenic amines such as dopamine, serotonin and octopamine (OA), the invertebrate equivalent of noradrenalin, regulate synaptic signaling in all multicellular species. Previous work has implicated both OA and the neurotransmitter glutamate as essential for egg laying (oviposition) in *Drosophila;* however the underlying mechanisms remain obscure. We are investigating how OA and glutamate regulate the muscles of the reproductive system and the manner in which octopaminergic and glutamatergic pathways interact. Using the genetically encoded calcium indicator GCaMP as an indicator for muscle contraction and neuronal activity, we show that OA causes sustained contractions in the lateral oviducts after a short delay, whereas glutamate causes an immediate but brief contraction

in the common oviduct. We also show that OA neurons at the tip of the abdominal ganglion regulate movements of the sperm storage organs and the uterus. Additional data suggest that up to three distinct OA pathways may contribute to the regulation of oviposition, and appear to be activated in a precisely coordinated temporal pattern. Interestingly, we observe two different firing patterns in both the octopaminergic and glutamatergic neurons: low amplitude tonic spikes and high amplitude bursts that could represent phasic firing. Exogenous application of glutamate increases the frequency of the tonic pattern in the OA cells, suggesting a potential regulatory pathway. Ongoing experiments will further define the mechanisms by which OA and glutamate interact to regulate the egg laying circuit and have the potential to uncover fundamental principles of aminergic neuromodulation.

423C Circadian rhythm affected by temperature control of *Drosophila melanogaster* *Summer Gul*, Changyoon Kim, Youngseok Lee. BIOFERMETATION AND BIOFUSION TECHNOLOGY, KOOKMIN UNIVERSITY, SEOUL, South Korea.

Circadian rhythms are optimized among most animals from flies to humans in order to synchronize biological functions and complex behaviors within 24 hours time period. Restricted to temperature control parameter, temperature sensors in the central nervous system might regulate internal biological clocks with external temperature shifts. By using *Drosophila melanogaster* as a model organism, we aimed to identify temperature regulating genes and to investigate possible underlying molecular mechanisms which may be homologous or similar to temperature controlled genes involved in humans' circadian rhythms. For dissecting temperature regulating system, we have previously performed a genome-wide temperature preference assay (Lee et al. 2005). For secondary screening, we selected out about 200 candidate genes through human homology and phenotypes from initial candidates. We finally chose 20 genes for further study. We will provide the prospective of novel genes in temperature regulating system.

424A Translational profiling of the Drosophila head fat body yields insights into the function of the adipose tissue clock *Amy Yu*, Yanmei Huang, F. Rob Jackson. Neurobiology, Tufts University Medical School, Boston, MA.

Circadian clocks are resident in a wide variety of tissues. While central brain clocks have been well studied, less is known about how peripheral clocks govern cell type specific programs of rhythmic gene expression (cycling). Genome-level circadian profiling of cell type specific gene expression has been hampered by the difficulty of isolating RNA from a single clock cell type. Dissection studies suggest that clocks do indeed have cell type specific functions. However, approaches based on physical separation of different cell types are inherently limited. Our lab has adapted translating ribosome affinity purification (TRAP) to selectively isolate ribosome-associated RNAs from specific clock cell types of interest. In TRAP, a GFP-tagged ribosome is selectively expressed in the target cell type, allowing immunoprecipitation of associated mRNA. Previously, our lab used TRAP with a tim-GAL4 driver to express a GFP-tagged ribosome in all clock cells of the Drosophila head. This allowed us to detect novel cycling mRNAs that are expressed at high levels in non-clock cells, and had thus been missed in studies using bulk tissue.

We are now using TRAP to profile individual clock cell types of the fly head with the goal of identifying cell type specific gene cycling. One project focuses on circadian profiling of the fly head fat body, which is a complex organ that shares functions with mammalian adipose tissue and liver and secretes a variety of signaling molecules. Via TRAP and bioinformatic analysis, we have identified approximately 450 genes that cycle in the fly head fat body. Enrichment analysis across timepoints suggests that approximately 140 of these cycling genes are expressed primarily in the fat body. A number of these genes have not been previously shown to cycle. Many of these fat body specific cycling genes cluster in functional groups related to behavior, including aggressive behaviors. Studies are currently underway to confirm that cycling of these genes depends specifically on the fat body clock.

425B Cellular and molecular dissection of cold nociception in *Drosophila* *Atit Patel*, Dustin Moon, Nathaniel Himmel, Daniel Cox. Neuroscience Institute, Georgia State University, Atlanta, GA.

Diverse organisms utilize thermoreceptors to detect and respond to noxious thermal stimuli. Recently, the basic cellular and molecular mechanisms underlying noxious cold perception has been identified. Class III (CIII) multidendritic (md) sensory neurons are specifically activated by cold and optogenetic activation of these neurons is sufficient to elicit the cold-evoked contraction (CT) behavior. Moreover, blocking synaptic transmission in CIII neurons inhibits CT behavior. Thus CIII neurons are multimodal with roles in noxious cold detection and gentle touch mechanosensation. To clarify how these multimodal sensory neurons distinguish between noxious cold and innocuous gentle touch, we developed and implemented an optogenetic dose response assay to determine whether CIII-mediated behavioral output is dependent upon activation levels. We demonstrate that gentle touch mediated head withdrawal behavioral responses are due to low dose opto-activation of CIII neurons, whereas noxious cold evoked CT responses are due to high dose CIII opto-activation. To further characterize multimodality of CIII md neurons, we utilized CaMPARI analyses to visualize neuronal activity patterns along the length of the larval body as a function of sensory stimulus. Cold evoked CT results from significantly higher CaMPARI photoconversion in CIII md

neurons relative to innocuous light touch. These data indicate that noxious cold more strongly activates CIII neurons compared to light touch revealing that CIII-mediated multimodal behavior is dependent upon activation levels. To initiate an investigation into cold nociceptive neural circuitry, we have conducted live, *in vivo* imaging of cold or optogenetic activation of CIII neurons measuring evoked calcium responses throughout the larval ventral nerve cord (VNC) via GCaMP6 and CaMPARI imaging. These analyses reveal that noxious cold stimuli are transmitted down CIII axons and initially progress from a posterior-to-anterior direction within the VNC followed by rapid lateral propagation. Based upon the cold-evoked neural activity patterns observed in the VNC, we have begun dissecting the second order interneuron populations that participate in this circuit via synaptic blockade and optogenetic activation strategies. Moreover, we have developed *in vivo* preparations for visualizing cold-evoked neural activation of motorneurons and postsynaptic muscle in this system. Collectively, we demonstrate how *Drosophila* larvae process distinct stimuli using multimodal neurons that produce unique behavioral outputs via complex nociceptive thermosensory and mechanosensory circuits.

426C CNS glia in adult *Drosophila* alcohol sensitivity *Kristen Lee*[1], Michael Grotewiel[2]. 1) Neuroscience, Virginia Commonwealth University, Richmond, VA; 2) Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA.

Studies in mammals are beginning to identify central nervous system (CNS) glia functional responses to alcohol administration (i.e. how glia respond to alcohol). Very few studies in any species, however, have explored the causal relationship between glial cell function and alcohol-related behaviors. To determine if glia are fundamental to the CNS response to alcohol, we are investigating how glia influence behavioral responses to alcohol by (i) determining if (presumed) global disruption of CNS glial cell function alters alcohol sedation sensitivity and (ii) exploring the influence of known molecular-genetic pathways within glia on alcohol sedation sensitivity. To investigate these phenomena, we are assessing ethanol sensitivity in flies with constitutive (via repo-Gal4) or adult-induced (via RU486 and 7293-1GS-Gal4) expression of a variety of transgenes that overexpress, block or knockdown the function of genes known to be active in CNS glia. To date, we have found that constitutive expression of RNAi against the genes axo, JhI-21, nemy and ent2 in CNS glia increases sensitivity to alcohol sedation. These RNAi results were specific to glia, not neurons, and did not change internal ethanol levels in the flies. Additionally, we have found that increasing oxidative stress (via RNAi-mediated knockdown of cytoplasmic Sod1) in CNS glia during adulthood reduces alcohol sedation sensitivity. Our data suggest that CNS glia act through specific molecular-genetic pathways to both protect against alcohol sedation and promote alcohol sedation sensitivity. We propose that CNS glia are fundamentally and dynamically involved in nervous system response to alcohol and are important for the ability of the nervous system to withstand alcohol insult.

427A Characterization of a novel mediator of nociception in adult *Drosophila melanogaster* *Bu-Guk Pil*, Mackenzie Hale, Sarady Merghani, Amanda Crocker. Neuroscience Program, Middlebury College, Middlebury, VT.

Noxious stimuli produce avoidance behaviors from paramecia to humans, yet one of the most commonly used noxious stimuli to train animals, electric shock, is poorly understood on a circuit level. This is particularly relevant in *Drosophila* where an electric shock has been used for over 40 years to elicit avoidance behavior to benign stimuli and study the mechanisms of memory formation. While we know that eventually dopamine neurons carry the aversive signal into the Mushroom Body (an important structure for memory formation), little is known about the genes involved in shock detection and circuitry to the dopamine neurons. A previous study of the gene expression changes following long-term memory led us to investigate the role of *NinaA* in memory formation. We quickly discovered that animals with decreased levels of *NinaA* failed to avoid electric shock while still maintaining their jump response to it. To fully characterize *NinaA*'s role in nociception we developed an assay which visually tracks the animals as they move on an electrified grid. We also determined whether *NinaA* played a more prominent role in nociception in general by looking across different assays assessing high and low temperature avoidance. NinaA is known to be important for trafficking *Rhodopsin 1 (Rh1)* in the eye. *Rh1* is also involved in temperature preference leading us to hypothesize that NinaA is acting through *Rh1*. Interestingly, we find that *NinaA* defects in shock perception are not isolated, they also show deficits in response to both high and low heat stress and this appears independent of *Rh1*. This is intriguing because previous work argues that temperature nociception is separate from shock nociception. Overall this work demonstrates that in nociception, particularly for a non-ethologically relevant stimuli such as shock, there is an overlap in genes and potential pathways.

428B Genes Underlying Behavioral Immunity Identified by Genome-Wide Association *M. Martin*, T. Schlenke. Department of Entomology, University of Arizona, Tucson, AZ.

Humoral immune mechanisms such as the production of antimicrobial peptides, and cellular immune mechanisms such as phagocytosis, are often considered to be synonymous with the immune system. However, our conceptualization of the immune system is changing to encompass other ways animals defend against pathogens and parasites. Behaviors that help an organism avoid or treat an infection constitute behavioral

immunity. *Drosophila* larvae are commonly parasitized by wasps in the genus *Leptopilina* with rates up to 80% in natural populations. Upon forced cohabitation in the lab, adult females will reduce the number of eggs laid by ~50%, presumably as an adaptive response to wasp presence. We performed a genome-wide association study using the *Drosophila* Genetic Reference Panel (DGRP) to examine the underlying genetic mechanisms of wasp-induced oviposition reduction. Across 177 DGRP lines sampled, wasp response varied with a range of 99% reduction in egg lay to a 39% increase in egg lay (average response of 45% reduction in egg lay). We identified 11 SNPs and 13 candidate genes associated with the observed variation. Some candidates have known roles in the perception of smell (*SKIP*), oogenesis (*mos*, *Btk29A*), and nervous system development (*CG13001*) while others are expressed only in the male reproductive tract (*Sfp26Ac*, *CG43185*), perhaps an indication of sexual conflict. Functional validation using RNAi and mutant stocks will further clarify the role of each candidate gene.

429C Parallel screens in *Drosophila* and human cell lines to identify modifiers of tau accumulation *H. Chester*, J. Kim, M. Avalos, B. Bleiberg, I. Al-Ramahi, M. de Haro, T. Gallego, H. Zoghbi, J. Botas. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Alzheimer's disease (AD) is the most common cause of dementia worldwide and the 6th leading cause of death. Despite its prevalence, there is currently no cure for AD. Two neuropathological hallmarks of AD are extracellular plaques and intracellular neurofibrillary tangles, which are composed respectively of amyloid-β and hyperphosphorylated tau. Accumulation of these two proteins contribute to the widespread death of cholinergic neurons. The presence of tau tangles strongly correlates with the severity of neurodegeneration in AD and has also been implicated in a variety of other neurodegenerative disorders, collectively termed tauopathies. The results of recent studies suggest tau as a driving force behind AD pathology and decreasing tau levels as a potential therapy. Using a *Drosophila* model to identify genetic modifiers of tau levels allows us a high throughput method to study the effect of modifier genes on both protein accumulation and tau-induced neuronal dysfunction. A parallel screen of approximately 4000 genes in both Daoy cells overexpressing tau and a *Drosophila* model expressing human tau generated a list of 88 candidate genes that when knocked down, decreased tau levels in both models. Characterization of these genes provides greater understanding of pathways that may play a role in AD pathogenesis.

430A p62/SQSTM1 is required for neurotoxicity in a *Drosophila* model of C9orf72-ALS. *KM Cunningham*, K Zhang, TE Lloyd. Neurology, Johns Hopkins University School of Medicine, Baltimore, MD.

In 2011, a GGGGCC hexanucleotide repeat expansion (G4C2 HRE) in an intron of the C9orf72 gene was identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), common neurodegenerative diseases. One of the pathological hallmarks of C9-ALS is the presence of cytoplasmic protein aggregates colocalized with the autophagy receptor p62/SQSTM1. Interestingly, mutations in p62/SQSTM1 are also a rare genetic cause of ALS through an unclear mechanism. In a Drosophila model of C9-ALS expressing $(GGGGCC)_{30}$, we have found that p62 is upregulated and forms large aggregates in motor neurons. p62 plays a key role in autophagy by binding ubiquitinated proteins and delivering them to the autophagosome for degradation via the lysosome. Surprisingly, we find that knockdown of p62 rescues degeneration in the fly eye and in motor neurons. Through genetic and pharmacologic upregulation of autophagy, the ubiquitin-proteasome pathway, and chaperones, we are able to rescue neurodegeneration caused by G4C2 expression. Because we have also found that nucleocytoplasmic transport is disrupted in $(GGGGCC)_{30}$ flies, we are investigating the relationship between defects in protein degradation and nucleocytoplasmic transport disruption. We propose that C9orf72-HRE expression causes dysregulation of protein folding and degradation leading to cytotoxic protein aggregation, and that this is rescued by aggregate clearance through genetic and pharmacological upregulation of chaperones, autophagy, and the ubiquitin-proteasome system. This study suggests that drugs targeting proteostasis pathways may have therapeutic potential for C9orf72-mediated ALS and FTD.

431B Effects of *insomniac* Knockdown on Alzheimer's-like Pathology in $A\beta_{42}$-expressing *Drosophila* *Jerry DeWitt*[1], Sarah Deleon[1], Hana Chuang[1], Thomas Finn[1,2], Hannah Monday[3], Jeremy Lee[1]. 1) Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA; 2) Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA; 3) Department of Neurobiology, Albert Einstein College of Medicine, Bronx, New York.

Alzheimer's disease (AD) is characterized by accumulation and aggregation of β-amyloid (Aβ) peptide resulting in neuronal loss. Soluble oligomers of Aβ, especially $A\beta_{1-42,}$ are thought to be the most toxic species and the primary cause of the neurodegeneration and memory loss observed in AD patients. Evidence in mice indicates that, during sleep, neuronal metabolites, including Aβ, are removed from the CNS at higher rates than during wakefulness (Xie, et al. 2013). Additionally, clinical studies show a correlation between AD pathology and sleep deprivation (Spira, et al., 2013) suggesting that sleep deprivation may accelerate AD pathology by reducing the clearance of neurotoxic forms of Aβ.

We have developed a *Drosophila* model for studying the relationship between sleep and AD pathology. The *Drosophila* AD model we utilize expresses $A\beta_{42}$ in the CNS and shows reduced longevity, abnormal behavior, and accumulation of $A\beta_{42}$ aggregates in the CNS. To study the effects of sleep deprivation on AD pathology, we express shRNA specific for *insomniac* in our AD model *Drosophila*. The Insomniac protein is involved in the regulation of the sleep-wake cycle in *Drosophila,* and knockdown of *insomniac* induces sleep deprivation. We have previously shown that $A\beta_{42}$-expressing flies in which *insomniac* is knocked down have a shorter mean lifespan than $A\beta_{42}$-expressing flies without *insomniac* knockdown. Knockdown of *insomniac* on its own has no effect on longevity. We report here on experiments to determine whether knockdown of *insomniac* exacerbates other aspects of AD-like pathology in *Drosophila*. Since $A\beta_{42}$-expressing flies exhibit defects in negative geotaxis, we utilize RING assays to determine whether sleep deprivation exacerbates these Aβ-induced deficits.

432C Effects of Estriol on Aβ-induced Pathology in a *Drosophila* Model of Alzheimer's Disease *Shiron Drusinsky*[1], Sarah Jane Long[1], Thomas Finn[1,2], Anakarina Lance[1], Jeremy Lee[1]. 1) Department of Molecular, Cell, & Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA; 2) Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA.

Alzheimer's disease (AD) is characterized by amyloid plaques, which are composed of the amyloid-β peptide (Aβ) that accumulates in the brains of affected individuals. Aβ adopts a variety of structural conformations, including insoluble high molecular weight plaques and soluble, low molecular weight toxic oligomers, suggesting that the structure of Aβ aggregates relates to its toxicity. Additionally, small molecules, found intracellularly and extracellularly, have been shown to interact with and alter the structure of Aβ aggregates. Research has shown that estrogen-related hormones, particularly estriol, both destabilize previously formed Aβ fibrils and inhibit their formation *in vitro*; this may, in turn, affect Aβ's toxicity. However, it is not yet clear the extent to which estriol affects Aβ toxicity *in vivo*.

In this study, we aim to elucidate the effect of estriol on $A\beta_{42}$ neurotoxicity *in vivo,* by using AD model *Drosophila* that express human $A\beta_{42}$ in their CNS. These flies show a reduction in longevity, behavioral deficits, and formation of Aβ aggregates in their CNS. In our experiments, AD model *Drosophila* are fed standard fly food with estriol added and compared to AD model *Drosophila* fed standard diets without added estriol. The lifespan of these flies is measured to determine the effect of estriol on $A\beta_{42}$-induced reduction in longevity. The concentration of estriol for feeding in these experiments was 500μM, the highest concentration of estriol we tested that did not affect the longevity of wild-type *Drosophila*. Western blotting in fly brains will also be done to determine whether estriol affects Aβ levels or its aggregation *in vivo*.

433A Profiling the brain transcriptome and proteome in a *Drosophila* model of Alzheimer's disease *Caiwei Guo*[1], Hari Krishna Yalamanchili[1,2], Eric Dammer[3], Qiudong Deng[3], Yi-Chen Hsieh[1], Philip De Jager[4,5,6], Nicholas Seyfried[3], Zhandong Liu[1,2], Joshua M. 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) Emory University School of Medicine, Atlanta, GA; 4) Harvard Medical School, Boston, MA; 5) Brigham and Women's Hospital, Boston, MA; 6) Broad Institute, Cambridge, MA.

Alzheimer's disease (AD) neuropathology is characterized by extracellular amyloid plaques and cytoplasmic neurofibrillary tangles, comprised of aggregated Aß peptide and microtubule associated protein Tau, respectively. In current models of AD pathogenesis, Tau is implicated as a key mediator of neurodegeneration but the underlying molecular mechanisms remain incompletely understood. *Drosophila* AD models based on pan-neuronal expression of human Tau (*ELAV>Tau*) recapitulate age-dependent neurodegeneration, and have facilitated mechanistic dissection in Tauopathies. In order to elucidate novel mechanisms, we have performed parallel deep RNA sequencing and mass spectrometry-based label free proteomics on *ELAV>Tau* or control fly heads prepared at 1-, 10-, and 20-days from triplicate samples. Bioinformatic analyses characterized Tau-associated differentially-expressed transcripts and proteins, as well as altered functional pathways, based on gene set enrichment analysis and weighted gene co-expression network analysis. Among up-regulated genes, Tau significantly activates transcripts and proteins with roles in proteolysis (e.g. *Damm*) and heat shock / protein folding responses (e.g. *Hsc70-1*). Conversely, Tau triggers reductions in transcripts and proteins with roles in redox metabolism (e.g. *Cyp4p1*) and translation (e.g. *eIF-4B*). Our results also suggest a discordance among differentially-expressed genes based on transcriptomic versus proteomic profiles, possibly due to differences in assay sensitivity as well as divergent cellular mechanisms for regulation of transcript and protein levels. We are currently validating selected targets/implicated pathways and interrogating complementary human brain transcriptomic and proteomic datasets for consistent changes in association with Tau pathology. Our unique dataset, including paired transcriptome and proteome from a widely used *Drosophila* model, will be a powerful resource for future investigations of Tau-induced neurodegeneration in AD.

434B Light-Dependent Endolysosomal Defects in a Photoreceptor Model of Alzheimer's Disease Michelle Smith, Jillian Wothe, *Adam Haberman*. Biology Department, University of San Diego, San Diego, CA.

Alzheimer's Disease (AD) is a neurodegenerative disease which causes progressive neuronal dysfunction and death. AD pathology has been linked to accumulation of amyloid and tau proteins. We have expressed amyloid β42 in photoreceptors to analyze cellular defects caused by amyloid accumulation. We find that exposure to light increases neurodegeneration in amyloid-expressing photoreceptors. Light exposure normally induces endocytosis and lysosomal degradation of Rhodopsin, but Rhodopsin accumulates in intracellular puncta in amyloid-expressing photoreceptors exposed to light. We propose that photoreceptors are especially sensitive to endolysosomal defects when exposed to light, due to bulk endocytosis of Rhodopsin. This characteristic of photoreceptors is relevant to understanding the more than 100 research articles that have used photoreceptors to study models of neurodegenerative disease. To test this theory, we blocked the formation of Rhodopsin by raising flies without Vitamin D, which dramatically rescued photoreceptor degeneration. We are currently preparing to block the formation of action potentials in amyloid-expressing photoreceptors by co-expressing an inward-rectifying potassium channel; this will differentiate between degeneration caused by Rhodopsin endocytosis and that caused by neuronal activity. We are also characterizing the endolysosomal defect in amyloid-expressing photoreceptors. To identify the organelles containing Rhodopsin accumulations, we are performing colocalization studies using antibodies for endosomes, lysosomes, and autophagosomes. We have also designed an *in vivo* pulse-chase assay to quantify the extent of endolysosomal defect in these cells. We will express a lysosome-targeted GFP specifically in photoreceptors under the control of a heat shock promoter. By quantifying the rate of degradation of GFP, we will determine how severely endolysosomal function is impaired by amyloid in these cells, with and without exposure to light.

435C BMP signaling improves locomotion and survival in a *Drosophila* model of Amyotrophic Lateral Sclerosis (ALS) *Aaron Held*, Paxton Major, Diane Lipscombe, Kristi Wharton. Brown University, Providence, RI.

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease with a prevalence of 3.9 cases per 100,000 people. The motor function of a patient rapidly declines after onset, typically causing death due to respiratory failure within 3-5 years. Autopsy studies show significant degeneration and death of motor neurons along with abnormal motor neuron-muscle connections that likely causes a patient's loss of motor function. BMP signaling plays important roles in normal motor neuron and muscle growth, and increases synaptic strength at the neuromuscular junction (NMJ), making it a potential candidate for slowing neurodegeneration in ALS. Our study demonstrates that BMP signaling is capable of improving NMJ structure and function in a *Drosophila* knock-in model of ALS. ALS-model animals expressing the BMP5/6/7 ligand, Gbb, have improved locomotor function and improved survival. Cell autonomous expression of an activated BMP receptor, SaxA, suggests that BMP signaling can act within motor neurons and upstream interneurons to improve locomotion phenotypes, but only the expression of SaxA in interneurons improves survival. The expression of the Rac GEF Trio, a target of BMP signaling that increases synaptic growth, in interneurons also substantially improves ALS-animal survival. Our data strongly suggest that BMP signaling within multiple cell types may be capable of improving motor function in ALS. Future studies will focus on identifying the targets of BMP signaling that are important for rescuing ALS-phenotypes in both motor neurons and interneurons.

436A A new *Drosophila* lysosomal protein with functions in stress resistance and neuroprotection in Parkinson disease models *A.-R. Issa*[1], J. Sun[1], C. Petitgas[1], G. Matassi[2], B. Chérif-Zahar[1], S. Birman[1]. 1) Genes Circuits Rhythms and Neuropathology, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, Paris, France; 2) Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy.

Autophagy is an intracellular process that delivers cytosolic components to lysosomes for degradation and recycling. Although autophagy is typically triggered by nutrient starvation, this process can also counteract the accumulation of protein aggregates or damaged organelles induced by stress, ageing and various human disease conditions. Alterations in autophagy mechanisms, such as macroautophagy and chaperone-mediated autophagy (CMA), have been described in Parkinson disease (PD), the second most common age-related neurodegenerative disorder. The CMA machinery depends on LAMP-2A, a lysosomal transmembrane glycoprotein involved in the translocation of selected cytosolic proteins into the lysosomal lumen. A major PD-associated protein, α-synuclein, is known to be a CMA substrate. Because LAMP-2A is lacking in invertebrates, it is generally considered that CMA does not occur in *Drosophila*. Here we observed that human LAMP-2A expression in fly neurons increased resistance to nutrient starvation and oxidative stress. We then identified a distantly related *Drosophila* lysosomal protein, named Lome, whose level is increased by prolonged starvation, and which shows quite similar neuroprotective properties compared to LAMP-2A. Either LAMP-2A or Lome prevented the age-related locomotor deficits and oxidative damages induced by transgenic expression of mutant human α-synuclein in *Drosophila* neurons, and, importantly, both proteins increased α-synuclein degradation in these conditions. In healthy flies, neuronal expression of either Lome or LAMP-2A did not extend lifespan but prolonged locomotor ability during ageing. Overall, this work provides

evidence for the existence of an autophagy pathway stimulated by either the endogenous protein Lome or human LAMP-2A that efficiently protects against oxidative stress and PD-inducing factors in *Drosophila*.

437B ***Presenilin*** **and** ***Nicastrin*** **knockdon in adult neurons of** ***Drosophila*** **causes age-dependent neurodegeneration, behavioral deficits and shrotend lifespan**

Jongkyun Kang[1], Sarah Shin[1], Norbert Perrimon[2,3], Jie Shen[1,4]. 1) Department of Neurology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA; 4) Program in Neuroscience, Harvard Medical School, Boston, MA.

Mutations in the *Presenilin* genes are the major genetic cause of Alzheimer's disease. Presenilin and Nicastrin are essential components of γ-secretase, a multi-subunit protease that cleaves single-pass transmembrane proteins, such as the Notch receptors. Genetic studies in mice demonstrated that Presenilin is essential for neuronal survival during aging, but the underlying molecular mechanism is unknown. The mammalian and *Drosophila* genes encoding γ-secretase components are well conserved; thus, fruit flies provide a powerful genetic system that permits large-scale screening to identify physiological substrates of γ-secretase. Here, we generated multiple lines of shRNA against *Presenilin* (*Psn*) or *Nicastrin* (*Nct*), resulting in 80-90% reduction of mRNAs, and confirmed that expression of these shRNAs in the wing disc indeed leads to notched wings. Interestingly, *Psn* or *Nct* knockdown (KD) in neurons causes early lethality, severe climbing defects and rough eye phenotypes. Furthermore, selective *Psn* or *Nct* KD in adult neurons using the *elav-gal4* and *tubulin-gal80^{ts}* system results in climbing defects, age-dependent neurodegeneration, increases in apoptosis and shortened lifespan, and these phenotypes are more severe in *Psn/Nct* double KD flies. These fly phenotypes bear striking resemblance to age-dependent neurodegeneration, increased apoptosis and earlier mortality of postnatal forebrain-restricted *Presenilin* or *Nicastrin* conditional knockout mice. These findings demonstrate that similar to their mammalian orthologues, *Drosophila* Psn and Nct are essential for neuronal survival and normal lifespan, suggesting that these *Psn* and *Nct* conditional KD flies are powerful tools for identifying molecular targets of γ-secretase that may be further explored as novel therapeutic targets of Alzheimer's disease.

438C Synthetic lethal screen in ***Drosophila*** **reveals connections between Huntington's disease and ALS**

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Neurodegenerative disorders such as Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS) lead to progressive loss of neurons that affect motor function. Genetic screens and Genome Wide Association Studies identified abundant genes that influence neurodegeneration. However, it remains largely elusive of how neuronal cell death is induced. To identify strong genetic modifiers that play key roles in HD and ALS, we used a synthetic lethal screen in *Drosophila*. Synthetic lethality represents essential interactions where co-expression of two mutated genes results in lethality. We report *Drosophila* alleles that are synthetic lethal with polyglutamine repeats associated with HD and hexanucleotide repeats associated with a form of ALS. Our data suggest that HD and ALS share common mechanisms of neurotoxicity.

439A Functional validation of an Alzheimer's disease susceptibility gene network

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Effective Alzheimer's disease (AD) therapies will come from improved understanding of pathogenic mechanisms, including the polygenic interactions underlying disease risk. Genome wide association studies (GWAS) have identified ~30 AD susceptibility candidate genes, including several which are similarly implicated in focal adhesion signaling. Knockdown of *Drosophila* gene orthologs for *PTK2B*, *CD2AP,* and *CASS4--Fak*, *cindr* and *p130CAS*, respectively--each enhance the neurotoxicity of human Tau, which aggregates to form neurofibrillary tangle pathology in AD brains. Further, Cindr and p130CAS physically interact and co-localize in the adult fly brain based on preliminary experiments, and all 3 proteins show reciprocal interactions in published work from other systems. Loss-of-function analysis of each gene in isolation reveals little or no evidence of age-dependent neurodegenerative changes in the adult fly brain, based on histology, electroretinogram neurophysiology, and survival analysis. However, mutant allelic combinations demonstrate robust genetic interactions suggesting that these genes may function coordinately to influence disease risk. In order to identify and validate additional components of this putative susceptibility network, we have partnered with the Acceleration Medicine Partnership-Alzheimer's Disease (AMP-AD) consortium, which has implicated many novel targets based on transcriptomic and proteomic profiles of human postmortem brains. In ongoing work, our *Drosophila* functional validation pipeline is systematically evaluating for neurodegenerative phenotypes following nervous system knockdown or overexpression of each conserved fly gene homolog; we are also testing for interactions in both Tau- and β-amyloid transgenic models of AD. In sum, our results

reveal a new AD susceptibility gene network, and future work promises to dissect the neuronal functions of molecular systems underlying disease risk and pathogenesis.

440B Large-scale functional analysis in HD *Drosophila* model of CAG-dependent genes identified in mouse models of HD *L. Li*[1,2], M.R. Avalos[1,2], B.A. Bleiberg[1,2], M.T. Gallegos[1,2], A.M. Perez[1,2], P. Langfelder[3], J. Rosinski[4], S. Horvath[5,6], X.W. Yang[5,7,8], I. Al-Ramahi[1,2], J. Botas[1,2]. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA; 4) CHDI Foundation/CHDI Management Inc., Princeton, NJ; 5) Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA; 6) Department of Biostatistics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA; 7) Center for Neurobehavioral Genetics, Semel Institute for Neuroscience & Human Behavior, University of California, Los Angeles, CA; 8) UCLA Brain Research Institute, University of California, Los Angeles, CA.

Huntington's disease (HD) is a dominantly inherited fatal neurodegenerative disorder with no effective treatments to date. Disease pathogenesis is due to selective degeneration of medium spiny neurons (MSNs) in striatum, and the pyramidal neurons (CPNs) in the cortex. The molecular cause of HD is the expansion of CAG repeats in the open reading frame of the gene that encodes Huntingtin (HTT) protein. Unaffected individuals have fewer than 36 repeats, whereas affected individuals have more than 36-39 CAG repeats. The length of the CAG repeat is inversely correlated with the age of HD onset.

Using deep mRNA sequencing, our collaborators at UCLA profiled the striatum, and cortex of mouse models of HD carrying one of six different CAG (Q) lengths. Together with proteomic studies, 790 proteins were revealed to have CAG length-dependent changes at both RNA and protein levels.

This large-scale transcriptomic analysis of expanded HTT identified a comprehensive set of genes and networks dysregulated in a CAG-dependent and age-dependent manner in mouse HD brains. A key challenge is to interpret the significance of these molecular alterations in the context of HD pathogenesis, which can be pathogenic or compensatory. Therefore, we are performing a comprehensive functional analysis of all the gene expression changes using mutant HTT-induced neuronal dysfunction readouts. Since these large-scale studies are not feasible in mice, we are using *Drosophila* models of HD in which we mimic or antagonize the expression changes observed in mouse brains and evaluate their impact on neuronal dysfunction in *Drosophila* using a behavioral readout. We will present a progress report of data obtained up to date.

441C Protein Network Extension to Identify Novel Modifiers of Huntington's Disease *Tarik S Onur*[1,2], Matthew Avalos[1,2], Benjamin Bleiberg[1,2], Tatiana Gallego[1,2], Ismael Al-Ramahi[1,2], Juan Botas[1,2]. 1) Molecular and Human Genetics, Baylor College Of Medicine, Houston, TX; 2) Jan And Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder with no treatments to prevent the onset of disease. HD is caused by a trinucleotide expansion, CAG, in exon 1 of the Huntingtin gene leading to the expansion of a poly-glutamine domain in the N-terminus of the protein Huntingtin (1). Reduction of mutant Huntingtin (mHTT) has been shown to be an effective strategy for delaying the onset of HD in animal models. Although direct targeting by oligonucleotide based therapies shows promise, the long-term efficacy and safety of these strategies in patients remains unclear(2). An alternative to directly targeting mHTT is to use drugs to target genes that can affect mHTT levels indirectly. Our laboratory screens genes that are deregulated during disease in human patients to identiy potetinal modifiers in various HD models, including *Drosophila*. We hypothesize that network extension will identify new potential therapeutic targets based on functional relationships with known modifiers of HD. Methods: We utilized the interactome database HumanNet to identify 491 functional protein interactors of a group of known, related modifiers of HD. To test these candidates I am using two *Drosophila* models of HD that neuronally express either a N-terminal (NT) or full length (FL) fragment of mHTT (5,6). Both FL and NT models have deficits in climbing that is the result of increased neuronal death due to an accumulation of mHTT (6). Female progeny of crosses between potential modifiers and HD flies are collected and tested in a custom machine that tracks the motor performance performance of individual flies in real time. Flies enter the climbing protocol following a period of aging, and are tested over ten days. Genes that are modifiers in this behavioral assay will also be knocked down in patient-derived fibroblasts to assess which of these modifiers reduce mHTT levels. Results: Ongoing screens conducted in our laboratory of random genes in the *Drosophila* genome for modifiers of HD have produced an approximately 18% hit rate. Of these genes that are modifiers of HD in *Drosophila*, we expect that 10% will reduce mHTT levels in Q111 cells, a cell line derived from striatal neurons of a humanized mouse model of HD. Conclusion: We can identify novel therapeutic targets to potetially treat HD by integrating pathway analysis with screening in multiple genetic models of HD. Work Cited (PMID): 1) 8458085 2) 251649893) 18381899 4) 21536720 5) 17984172 6) 18184562

442A Effects of genetic variation on retinal degeneration in *Drosophila* *Rebecca Palu*, Shani Chung, Clement Chow. Human Genetics, University of Utah, Salt Lake City, UT.

Retinitis pigmentosa (RP) is a retinal degenerative disease that is linked to mutations in many genes involved in diverse pathways. RP is also phenotypically heterogeneous; patients carrying identical causative mutations can differ substantially in their disease severity due to variable expressivity and penetrance. The extensive range of RP outcomes is thought to be due to cryptic genetic variation between individuals, but the genetic modifiers responsible for this variability are unknown. In previous work from our lab, a well-characterized *Drosophila* model of autosomal dominant RP ($Rh1^{G69D}$) was crossed to the *Drosophila* Genetic Reference Panel or DGRP. Eighty-four conserved candidate modifiers were identified, nearly half of which are involved in apoptosis. This is particularly striking, as apoptosis is a common target for RP therapy development. A common set of retinal degeneration modifiers that alter apoptosis may point to common therapeutic approaches relevant for many forms of retinal degeneration. To determine the degree to which these candidates are universally involved in different forms of retinal degeneration, we have crossed additional models of apoptosis-induced retinal degeneration into the DGRP. We have overexpressed *p53* or *reaper* in the developing eye using the *GMR-GAL4* driver. Similar to the RP model, eye size varies in response to these apoptotic models across the various strains of the DGRP. We see strong correlation in DGRP strains between the degeneration observed in the three models. We find that some of the candidate modifiers identified using these diverse retinal degeneration models are shared, while others are unique to each individual model. Knockdown of candidate modifier genes using RNAi reveal genetic interactions between modifier genes and the different retinal degeneration models, some of which specifically alter apoptotic pathways. Identifying a set of common natural genetic modifier genes that alter different forms of retinal degeneration will point to potential common therapeutic approaches.

443B A comparison of lifespan in two *Drosophila* models of Alzheimer's disease and the effects of expression of the human chaperone protein HSP70 *Erin F Patterson*, Kevin Hagy, Jeremy C Lee. Molecular, Cell & Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA.

Alzheimer’s disease (AD) is the leading cause of dementia and currently affects over 5 million Americans (CDC, 2016). It is known that both Tau neurofibrillary tangles and plaques composed of Aβ peptide are diagnostic of the disease, but the role of each in its pathogenesis are not well understood. Research has shown that soluble oligomers of Aβ-42 are particularly neurotoxic. Aβ is generated by amyloidogenic processing of APP, involving sequential proteolytic cleavage of APP by beta secretase, encoded by the BACE1 gene, and gamma secretase.

Previous studies suggest that overexpression of molecular chaperone proteins, including HSP70, could assist in prevention of misfolding of Aβ and/or its clearance. This could inhibit the formation of neurotoxic Aβ oligomers and delay or prevent the onset of AD symptoms (Lu et al., 2014). Our study aims to observe the effects of human HSP70 expression on the longevity of AD-model *Drosophila* and to compare this effect in two different *Drosophila* models of AD. In one model, human APP and human BACE1 are expressed pan-neuronally, allowing Aβ production by amyloidogenic processing of APP. In our other model, Aβ-42 is expressed directly pan-neuronally. In both models, expression of these human peptides has been shown to result in AD-like pathology, including shortened lifespan. We hypothesize that AD model flies that also overexpress human HSP70 have an increased lifespan as compared to AD-model flies that only express endogenous *Drosophila* HSP70.

Our data indicate that, of the two *Drosophila* models of Alzheimer's disease, the model with pan-neuronal expression of hAPP and hBACE1 is a stronger model. Flies expressing hAPP and hBACE1 have a significantly shorter lifespan than flies directly expressing Aβ-42. Our results also suggest that pan-neuronal hHSP70 expression extends the lifespan of both AD models.

444C Developing a *Drosophila* ALS model to reveal pathogenic mechanisms and test novel therapeutic approaches. *Sarah Perry*[1], Yifu Han[2], Dion Dickman[1]. 1) Neurobiology, USC, Los Angeles, CA; 2) USC Neurobiology Graduate Program.

Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative disease characterized by progressive motor neuron dysfunction, weakening of the neuromuscular junction (NMJ), and muscular atrophy. Hereditary ALS is strongly associated with intronic repeat expansion in the human *C9orf72* gene, and aggregation of toxic arginine-containing peptides is thought to play a role in ALS pathophysiology. Emerging evidence suggests that overexpression of disease-related *C9orf72* transgenes in *Drosophila* and other systems causes NMJ dysfunction akin to that observed in ALS. Although *C9orf72* models have been developed in flies, relatively little is known about how synaptic structure, growth, function, and plasticity is impacted in these models. We therefore systematically characterized these synaptic properties in a fly *C9orf72* model, utilizing the larval NMJ as a model synapse. We observe dramatic reduction in synaptic area and alterations in active zone structure, which corresponds to weakened synaptic transmission. Interestingly, postsynaptic receptors are upregulated, perhaps suggesting a compensatory response to reduced presynaptic release. However, transmission is only maintained at about half of *wild-type* levels. Next, we determined that *C9orf72* synapses still maintain the ability to homeostatically potentiate release following

pharmacologic challenge to postsynaptic receptors. In particular, when postsynaptic receptors are blocked by philanthotoxin application, presynaptic release doubles, similar to what is normally observed in *wild-type* synapses. We will present a series of experiments designed to activate these homeostatic mechanisms and restore proper synaptic strength in *C9orf72* synapses despite NMJ degeneration. Together, these studies will both reveal mechanisms of ALS-related synaptic dysfunction and test whether compensatory forms of endogenous homeostatic plasticity can ameliorate disease progression.

445A Ecdysone (Ec) signaling pathway activation can block Aβ42 mediated neurodegeneration *M. Riccetti*[1], N. Gogia[1], A. Sarkar[1], L. Payton[1], K. Moberg[2], M. Kango-Singh[1], A. Singh[1]. 1) Department of Biology, University of Dayton, Dayton, OH, United States; 2) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA, United States.

Alzheimer's disease (AD), a chronic neurodegenerative condition, exhibits characteristic neuropathology due to accumulation of extracellular Aβ42 protein. Currently, no proper early detection methods or cures exist for AD, but promising molecular genetic evidence is arising from studying the development of nervous tissue in model organisms like Drosophila melanogaster. In order to better understand the mechanism by which this disease progresses and its effects on nervous tissue, we misexpressed human Aβ42 in the developing eye of Drosophila. This stable transgenic line results in GMR-GAL4 driven UAS-amyloid-beta (GMR>Aβ42) mediated cell death in the eyes of nearly 100% flies at 29°C. We identified the Ecdysone (Ec) signaling pathway as a modifier of neurodegeneration caused by Aβ42 accumulation in the eye. Recent research has shown that the Ec signaling pathway modulates Hippo transcriptional activity in imaginal disc cells. The Ec coactivator Taiman (Tai) forms a unique transcriptional complex with the Hippo transcription factor Yki, altering expression of canonical Hippo targets and inducing transcription of germline stem cell factors in regions that have already differentiated. We proposed to a) Investigate if the Ec pathway can trigger cell proliferation machinery through induction of stem cell factors, and b) to investigate if the Ec pathway can block cell death machinery. Our data suggests that upregulation of the Yki-Tai transcription complex constituents does not induce germ cell-like growth in the GMR domain, but does block apoptotic cell death. We also found that Taiman upregulation leads to increased expression of canonical Hippo target Diap1, which subsequently blocks expression of the pro-apoptotic caspase Hid, rescuing Aβ42 mediated neurodegeneration in our Drosophila eye model. We propose to continue to understand the underlying molecular genetic mechanism responsible for Ec/Hippo-mediated rescue of Aβ42 mediated neurodegeneration to identify a novel protein interaction network for AD therapeutics.

446B Understanding the role of Wingless (Wg) signaling pathway in Amyloid-beta 42 (Aβ42) mediated neurodegeneration in Alzheimer's Disease *A. Sarkar*[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3,4]. 1) Department of Biology, University of Dayton,300 College Park Drive, Dayton, OH, USA; 2) Premedical Program, University of Dayton,300 College Park Drive, Dayton, OH, USA; 3) Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, Dayton, OH, USA; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN, USA.

Alzheimer's disease (AD), a common form of dementia and an age related progressive neurodegenerative disorder, manifests as memory loss and reduced cognitive ability. One of the hallmarks of AD is formation of the Amyloid-beta 42 (hereafter Aβ42) plaques, which triggers oxidative stress due to aberrant signaling and finally results in the death of neurons. However, the exact mechanism causing cell death is still not well understood. We misexpressed high levels of human Aβ42 protein in the developing fly retina, which mimics AD like neuropathology. In a forward genetic screen, we identified members of highly conserved Wingless (Wg) signaling pathway as modifiers of the Aβ42 mediated neurodegeneration. Misexpression of negative regulator of Wg like Shaggy kinase (*sgg*) or a dominant negative form of *Drosophila* T-cell factor ($dTCF^{DN5}$) or blocking Wg transport specifically by downregulating Porcupine (using $porcupine^{RNAi}$) rescued Aβ42 mediated neurodegeneration by reducing the number of dying cells and restoring the axonal targeting from the retina to the brain. We have developed a drug feeding regimen for flies and will test if we can use chemical inhibitors to block Wg signaling in neurons expressing high levels of Aβ42 and thereby prevent neurodegeneration in the *Drosophila* eye. This study can further help in finding out if Wg can be a good therapeutic target in our in vivo animal system. Furthermore, in order to determine the role of Wg in early vs late onset of AD, we have modulated our transgenic expression system to activate at different time points and will assess whether Wg is activated in all stages. The results from these studies will be presented.

447C The Use of *Drosophila melanogaster* as a model to Investigate the Underlying Cellular and Molecular Basis of TBI *Ivan Silva*, Taj Rai, Pedro Medina, Hector Villa. Biology, CSU San Bernardino, San Bernardino, CA.

Abstract: Each year in the United States, 1.7 million people experience a traumatic brain injury (TBI). A TBI is characterized by the severe impact to the cranial region in which the brain consequently slams against the inside of the skull. This can result in swelling and bruising of the brain, which can lead to decreased brain function. TBI is a significant health issue worldwide, yet the mechanism responsible for its complex pathologies remains largely unknown. Sustained TBI's have been shown to increase the likelihood of developing Alzheimer's (AD) and other

neurodegenerative diseases. Our research aims to identify some of the possible cellular and molecular mechanisms of TBI by utilizing the model organism *Drosophila melanogaster*. Two strains of *D. melanogaster* were used in this study, a transgenic Aβ 42 strain that expresses a 42 amino acid long peptide and a genetically matched 00C strain that serves as the control. The accumulation of amyloid-β (Aβ)42 peptide, the major component of senile plaques, has been hypothesized to be the primary event in AD pathogenesis. The TBI model for *D. melanogaster* consists of the use of a spring action apparatus that delivers a strong jolt of 0.1 sec. The experimental design included: 00C untreated flies, 00C treated with 5 daily TBI's, or with 10 daily TBI's, Aβ-42 untreated flies, Aβ -42 treated with 5 daily TBI's, or with 10 daily TBI's. Lifespan, cognitive ability (negative-geotaxis assay), cholinergic neuron viability as assessed by GFP expression, and cholinergic neuron quantity were analyzed to assess the effects of TBI. Although it was found that both Aβ-42 and the control showed the same inverse relationship between TBI treatments and lifespan, the Aβ-42 strain experienced a shorter lifespan than the control. Results from the climbing assay show the same trend for both strains in that increased daily TBI inducing treatments resulted in decreased climbing ability, however, each treatment group in the Aβ-42 strain showed a greater decreased climbing ability than that of the 00C strain. In terms of cholinergic neuron function, groups treated with ten daily TBI's experienced the sharpest decline. Neuronal function assessment indicates the Aβ-42 strain had a lower average and lower deviation in measured intensity of GFP expression, whereas the 00C strain had a higher average and higher deviation in measured intensity of GFP expression. In future studies, behavioral assessments could also be incorporated such as a sugar preference assay or odor avoidance assay as they might give us a better insight on the behavioral pathologies identified in humans.

448A Mechanism of neurodegeneration in CMT2C caused by TRPV4 mutations *Brian Woolums*[1], Jeremy Sullivan[2], Morgan Yang[2], Catherine Mamah[2], Charlotte Sumner[2], Thomas Lloyd[2]. 1) Pharmacology, Johns Hopkins University, Baltimore, MD; 2) Neurology, Johns Hopkins University, Baltimore, MD.

Dominant missense mutations in the gene encoding TRPV4 cause Charcot-Marie-Tooth disease subtype 2C, a motor predominant inherited peripheral neuropathy. Neuropathy causing mutations increase TRPV4 channel activity leading to increased intracellular calcium and cellular toxicity *in vitro*; however, the mechanisms of neurotoxicity are unknown. We have generated transgenic *Drosophila* that express either wild type human TRPV4 or a neuropathy causing variant, $TRPV4^{R269C}$. We observe that $TRPV4^{R269C}$ disrupts wing expansion when expressed in bursicon neurons, and perforated patch clamp analyses reveal that $TRPV4^{R269C}$ causes calcium-dependent neuronal hyperexcitability and membrane potential oscillations. These effects are reversed with a TRPV4 selective antagonist. Furthermore, we have performed an RNAi screen for modifiers of the $TRPV4^{R269C}$-mediated wing phenotype and have identified CaMKII as a potent suppressor of $TRPV4^{R269C}$. $TRPV4^{R269C}$ expression causes an increased frequency of calcium transients in larval dendritic arborization (da) neurons and markedly alters da neuron morphology, resulting in progressive synapse loss and diminished dendritic arborization. These morphological abnormalities are rescued by feeding larvae a TRPV4 antagonist. We are currently assessing whether knockdown of CaMKII or other modifiers rescue $TRPV4^{R269C-}$mediated phenotypes. Our data indicate that CMT2C is caused by TRPV4-mediated calcium influx and support the use of TRPV4 antagonists as potential treatments for this disease.

449B Tip60 increases histone acetylation levels and protects against neuronal apoptosis in the Aβ neurodegenerative *Drosophila* brain *Haolin Zhang*, Felice Elefant. Drexel University, Philadelphia, PA.

Apoptosis or programmed cell death is crucial in guiding the physiological development of individual cells and organs and is particularly important for brain development. Misregulation of this process leads to inappropriate induction of neuronal specific apoptotic cell death that has been shown to be a hallmark of certain progressive neurodegenerative diseases, one of which is Alzheimer's disease (AD). Apoptotic induced neural cell death in neurodegenerative diseases reveals active participation of appropriate histone acetylation homeostasis. Cases of reduced histone acetylation levels are found in the brains of animal models for multiple types of neurodegenerative diseases, including AD. We previously demonstrated that overexpression of the histone acetyltransferase (HAT) Tip60 blocks amyloid precursor protein (APP)-induced neuronal apoptosis by restoring histone acetylation balance in the *Drosophila* brain. However, whether Tip60 can also protect against more severe neurodegenerative conditions induced solely by human amyloid beta (Aβ) production that models AD stages specifically associated with plaque neurotoxicity remains to be explored. We have developed a new *Drosophila* model (Aβ42;Tip60) that allows us to modulate Tip60 HAT levels in *Drosophila* neural circuits under human Aβ-induced neurodegeneration conditions. Here we show that Tip60 and Tip60 mediated histone acetylation levels are significantly reduced in the neurodegenerative Ab *Drosophila* brain. We are currently utilizing our Aβ42;Tip60 fly line to test whether such Aβ-induced apoptosis and decreased histone acetylation in the *Drosophila* brain can be rescued by increasing Tip60 HAT levels as we hypothesize. We discuss the possibility that Tip60 may play an essential neuroprotective role in Aβ-induced neurodegenerative pathology.

450C PINK1-dependent Phosphorylation of PINK1 and Parkin Is Essential for Mitochondrial Quality Control *Na Zhuang*[1,2]. 1) National Institute of Biological Sciences, Beijing, China; 2) School of Life Sciences, Tsinghua University, Beijing, China.

Mitochondrial dysfunction has been linked to the pathogenesis of a large number of inherited diseases in humans, including Parkinson's disease, the second most common neurodegenerative disorder. The Parkinson's disease genes *pink1 and parkin*, which encode a mitochondrially-targeted protein kinase, and an E3 ubiquitin ligase respectively, participate in a key mitochondrial quality control pathway that eliminates damaged mitochondria. In the current study, we established an *in vivo* PINK1/Parkin-induced photoreceptor neuron degeneration model in *Drosophila* with the aim of dissecting the PINK1/Parkin pathway in detail. Using LC-MS/MS analysis, we identified Serine 346 as the sole autophosphorylation site of *Drosophila* PINK1, and found that substitution of Serine 346 to Alanine completely abolished the PINK1 autophosphorylation. Disruption of either PINK1 or Parkin phosphorylation impaired the PINK1/Parkin pathway, and the degeneration phenotype of photoreceptor neurons was obviously alleviated. Phosphorylation of PINK1 is not only required for the PINK1-mediated mitochondrial recruitment of Parkin, but also induces its kinase activity toward Parkin. In contrast, phosphorylation of Parkin by PINK1 is dispensable for its translocation, but required for its activation. Moreover, substitution with autophosphorylation-deficient PINK1 failed to rescue *pink1* null mutant phenotypes. Taken together, our findings suggest that the autophosphorylation of PINK1 is essential for the mitochondrial translocation of Parkin and for subsequent phosphorylation and activation of Parkin.

451A *Determining pathogenicity of variants observed in CMG/UDN patients and molecular mechanisms associated with mutations in CACNA1A* *X. Luo*[1], M. Wangler[1,2,3,4], S. Yamamoto[1,2,3], J. Rosenfeld[1], S. Lalani[1], T. Harel[1], RA. Lewis[1], L. Emrick[1], T. Lotze[1], M. Eldomery[1], ZC. Akdemir[1], J. Lupski[1], B. Lee[1], H.J. Bellen[1,2,3]. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital (NRI); 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston, TX.

Variants in *CACNA1A* [MIM 601011]*,* encoding the α-1A subunit of the neuronal P/Q type voltage gated Ca2+ channel, are known to cause ataxia and migraine. Triplet repeat expansions leading to polyglutamine tract expansions in the protein cause adult-onset spinocerebellar ataxia, type 6 [MIM 183086], whereas heterozygous variants (typically loss-of-function) cause childhood-onset episodic ataxia, type 2 [MIM108500]. Additionally, missense variants in the gene cause autosomal dominant familial hemiplegic migraines [MIM 141500]. Here, we report a patient with congenital, non-fluctuating ataxia, hypotonia, ophthalmologic abnormalities, and global developmental delay. The individual has a *de novo* missense variant, c.5018G>C/p.R1673P who was diagnosed via the Undiagnosed Disease Network and the Center for Mendelian Genomics. The phenotype is much more severe than the ones observed in the other patients.

The R1673P variant alters an arginine residue within the S4 transmembrane α-helix in the fourth domain, changing the pattern of positive charges within the voltage sensor. The pattern of positive charges is also altered by other severe pathogenic variants, suggesting that severe clinical phenotypes result from the disruption of this specific region. To explore the functional consequences of these variants, we generated a loss-of-function (LOF) T2A-Gal4 allele in the homologous gene, *cacophony (cac)* in *Drosophila.* The *cac* T2A-Gal4 gene trap line recapitulates the lethal phenotype previously observed in *cac* null mutant flies and fails to complement other *cac* alleles. Human variants are assessed through their ability to rescue the lethality of T2A-Gal4 mutant as well as the phenotypes associated with the loss of *cac* in *Drosophila* photoreceptors. These include neurodegeneration, synaptic transmission deficits and accumulation of autophagic vesicles. Our data suggest that *CACNA1A* can cause congenital non-fluctuating cerebellar ataxia and other severe neonatal presentations, expanding the spectrum of ataxias and other features associated with this important neuronal calcium channel gene. Functional studies in flies also provide further insight into the neuronal mechanisms of this disease spectrum and permit us to assess how the Ca++ channel functions.

452B Identification and characterization of novel modifiers for Spinal Muscular Atrophy *Graziadaniela Raffa*[1], Paolo Maccallini[1], MariaLaura DiGiorgio[1], Francesca Bavasso[1], Emanuela Micheli[1], Fabian Feiguin[2], Stefano Cacchione[1], Brian McCabe[3], Elia Di Schiavi[4]. 1) Sapienza University, Rome; 2) ICGEB, Trieste; 3) EPFL, Lausanne; 4) CNR, Naples.

SMA is a hereditary neuromuscular disorder caused by a reduced SMN dosage, which causes motor neurons loss in the spinal cord and muscle atrophy; SMN is the main component of the SMN complex, which acts as a chaperone for the assembly of the small nuclear ribonucleoproteins (snRNPs). Because the pathways involving SMN are highly conserved during evolution, we sought to identify conserved proteins that modulate SMN function. We developed a novel fly model for SMA, based on RNAi-mediated knockdown of Smn in neurons. In addition to the expected locomotion defects, we found that pan-neuronal depletion of Smn induces severe wing expansion defects and a failure to retract the ptilinum head muscle, necessary during eclosion. The unexpanded wings and unretracted ptilinum phenotypes indicate that Smn is essential in neurons to ensure completion of post-eclosion programs and

can be conveniently exploited to screen for genetic modifiers of Smn. We have found that WDR79/TCAB1 and TGS1, two proteins involved in the Smn pathway, are necessary for proper locomotion behavior in flies. Notably, WDR79/TCAB1 or TGS1 overexpression ameliorate the Smn mutant phenotypes, thus behaving as Smn modifiers. We will discuss the functional conservation of these genetic interactions in worms and humans and the impact of these findings on our understanding of SMA pathogenesis.

453C To investigate the impact of traumatic brain injury (TBI) in *Drosophila* models of ALS *Aditi Singh*[1,3], Lauren Gochenaur[1,3], Krishani Patel[1], Nandini Ramesh[1,2], Ian Casci[1,2], Udai B Pandey[1,2]. 1) Pediatrics, Children's Hospital of UPMC, Pittsburgh, PA; 2) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Neuroscience, University of Pittsburgh, Pittsburgh, PA.

Traumatic Brain Injury (TBI) is a syndrome that occurs when the brain suffers a trauma from high impact or force collision and results in temporary dysfunction of normal brain activity. TBI can result from concussions, commonplace recreational play (i.e. soccer), and military service. TBI is linked with predisposed risk factors in neurodegenerative (ND) disorders like Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's disease. However, it remains unclear how TBI affects disease progression leading to mortality; specifically, how early exposure to TBI causes neurological symptoms.

To understand the long-term impact of TBI, we generated an model of TBI in *Drosophila melanogaster* larvae, a well-studied model organism. In order to understand how TBI exposure affects early stages of Drosophila, we treated first and third instar larvae with TBI at varying amounts of force and hit repetitions using a HIT device. We observed that the degree of mortality is directly proportional to the number of hit repeats and trauma to the *Drosophila* larva. We recorded mortality index for larvae 24- and 48-hours post-TBI, and lifespan of adults through an eclosion assay. We found that at higher angles both first and third instar larvae experienced higher percentages of mortality. Furthermore, higher numbers of repeated hits also induced higher percentages of mortality. Overall, we observed more profound impacts in both parameters in first instar larvae. However, first instar larvae were able to withstand hits at lower angles, lower amounts of force, and had longer life spans. Furthermore, we found that in adults expressing ALS-linking genes with pathogenic mutations in neurons exposed to multiple mild TBI events displayed similar mortality trends and reduced motility in climbing assays. Post-TBI adults displayed consistent changes in mortality and locomotor dysfunction; thus, suggesting that mild TBI might influence ALS symptoms in our fly models. We observed profound accumulations of high molecular weight ubiquitnated proteins in the animals expressing ALS-causing mutations along with TBI, suggesting that defective protein degradation pathway (e.g autophagy) may be responsible for the build-up of ubiquitnated proteins.

In summary, our larval and ALS-TBI models demonstrate effects on lifespan due to TBI and generates a new avenue to learn cellular and molecular mechanisms of trauma. It generates new tools for analyzing early onset of NDs e.g. ALS.

454A Defining the transcriptional & behavioral defects of KDM5 mutations associated with Intellectual Disability. *Sumaira Zamurrad*, Xingyin Liu, Julie Secombe. Genetics, Albert Einstein College of Medicine, Bronx NY, 10461.

KDM5 proteins are multi-domain transcriptional co-factors that function by recognizing and enzymatically altering specific histone modifications. There are four broadly expressed KDM5 orthologs in mammalian cells (KDM5A-D) and a single KDM5 in Drosophila. Emphasizing the importance of KDM5 proteins, KDM5A and KDM5B are over expressed in a number of metastatic cancer types, and loss of function mutations in KDM5A, B and C are found in patients with intellectual disability (ID). To-date, 31 mutations in KDM5C, 7 in KDM5B and 1 in KDM5A have been identified in patients with either syndromic or non-syndromic ID. However, the molecular mechanisms underlying the link between mutations in KDM5 family genes and cognitive phenotypes remain elusive.

As a first step in establishing *Drosophila* as a model system to understand KDM5-induced ID, we defined direct KDM5 target genes in adults, as this is the stage used to model cognitive disorders. By combining RNA-seq from kdm5 hypomorphic adults with anti-KDM5 ChIP-seq, we identified 2455 (put number here) direct targets. Among these were several genes previously implicated in neurological disorders such as fragile-X syndrome, Down syndrome, autism and Alzheimer's. Because patients with mutations in KDM5 genes show cognitive impairment, we used a behavioral assay in flies and found that the flies with reduced levels of KDM5 have impaired appetitive-olfactory associative learning. Based on these data suggesting that flies provide a good model to define the link between KDM5 and cognition, we have generated eight fly strains harboring mutations in fly KDM5 that are analogous to disease-associated missense mutations in human KDM5A or KDM5C. 7/8 of the ID mutants generated thus far are expressed at wild type levels re-enforcing that these mutations affect function and not protein stability. To-date, we have examined one ID mutant fly strain in more detail and found it to have a severe learning and memory defect. Preliminary brain staining show that this mutant also shows morphological defects in the mushroom bodies required for olfactory learning and memory. We are currently testing other ID allele fly strains for similar cognitive impairment using appetitive and aversive-odor association learning and memory assays. Transcriptional

analysis will be done by RNA-seq. These studies will allow us to define for the first time whether similar or distinct target genes are affected by disease-associated alleles and allow us to define the mechanistic link between KDM5 dysfunction and intellectual disability.

455B The Effect of *Rhodiola rosea* on a *Drosophila melanogaster* Model of Huntington's Disease *Jasmin Arabit*[1], Samuel E. Schriner[1], J. Lawrence Marsh[2], Mahtab Jafari[1]. 1) Pharmaceutical Sciences, University of California, Irvine, Irvine, CA; 2) Developmental and Cell Biology, School of Biological Sciences, University of California, Irvine, Irvine, CA.

Huntington's disease (HD) is a dominant, late-onset disease characterized by choreiform movements, cognitive decline, and personality disturbance. It is caused by a CAG repeat expansion in the HD gene encoding for the huntingtin protein (Htt) which functions as a scaffold for selective macroautophagy. Mutant Htt (mHtt) disrupts vesicle trafficking and prevents autophagosome fusion with lysosomes, thus deregulating autophagy in neuronal cells, leading to cell death. Autophagy has been described as a therapeutic target for HD, owing to the key role Htt plays in the cellular process. *Rhodiola rosea*, a plant extract used in traditional and integrative medicine in Europe and Asia, has been shown to attenuate aging in the fly and other model species. It has also been shown to inhibit the mTOR pathway and induce autophagy in bladder cancer cell lines. We hypothesize that *R. rosea,* by inducing autophagy, may improve the HD phenotype in a fly model. Flies expressing HttQ93 which exhibit decreased lifespan, impaired locomotion, and increased neurodegeneration will be supplemented with *R. rosea* extract, and the extract's effect on lifespan, locomotion, and pseudopupil degeneration will be determined. The objectives of this work will be to examine the possibility of using *R. rosea* as a preventive treatment for HD. These experiments will be completed in February 2017 and our results will be presented in our poster.

456C Phosphorylation of serine residues of mutant huntingtin contributes to metabolic function in Huntington's disease in *Drosophila* *Megha Chatterjee*, Namita Agrawal. Department of Zoology, University of Delhi, Delhi, India.

Along with the neurological manifestations, metabolic alteration is a well-recognized peripheral manifestation that can be witnessed as progressive weight change and skeletal muscle wasting in Huntington's disease (HD) patients. Phosphorylation of serine 13 and serine 16 of Huntingtin (Htt) affects the conformations, sub-cellular localization and clearance of Htt. Reports on HD mouse model and *in vitro* studies suggest that phospho-mimetic mutations at S13 and S16 impedes neuronal mHtt aggregation but preserved in phosphoresistant S13/16 mutations, which ultimately influences disease pathogenesis. Therefore, to further understand the influence of serine phosphorylation of mHtt on metabolic function in HD, we used transgenic *Drosophila* with truncated exon1 fragment where serines were modified either to aspartate (phosphomimetic or SD) or alanine (phosphoresistant or SA) with expanded 120Qs.

To evaluate relevance of SA and SD in formation of Htt aggregates in *Drosophila*, we monitored neuronal exon1 mHtt inclusions in eye imaginal disc of SD and SA larvae by immunohistochemistry. We detected neuronal aggregates in SA larvae whereas SD larvae show abrogation of aggregates. In accordance with the formation of aggregates in neurons, the viability of SA flies was also witnessed to be significantly reduced as compared to the SD flies. Evaluation of body weight in SA mutants displayed a progressive weight gain throughout the course of the disease. To get a better insight of the weight gain, the level of energy metabolites was estimated in SA and SD flies at different ages. Significant alterations in glycogen, trehalose, lipid and protein levels were observed in SA flies and interestingly SD flies were comparable to the control flies with unexpanded polyQ tract.

Our findings clearly demonstrate that serine 13 and 16 of mHtt are critical in disease pathogenesis in *in vivo* condition. We further propose that regulation of aggregates and metabolic activity might play a key role in modulation of HD pathogenesis by serine 13 and 16. Therefore, targeting these serine residues could be a potential strategy towards HD therapy.

457A Detrimental effects of mutant huntingtin beyond neurons: Alteration of metabolic activity in *Drosophila* *Priya Lakra*, Kumari Aditi, Namita Agrawal. Department of Zoology, University of Delhi, Delhi, India.

Abnormal expansion of a polyglutamine stretch (PolyQ) in the N-terminus region of huntingtin (Htt) protein leads to the devastating neurodegenerative Huntington's disease (HD). PolyQ expanded Htt is very well documented to be involved in selective neurodegeneration in HD. However, the deleterious effects of mutant Htt (mHtt) apart from neuronal degeneration are largely unknown.

We investigated the effect of truncated exon1 peptide of mHtt containing 120 glutamine residues in fat body exclusive of nervous system using transgenic *Drosophila* as a model. Interestingly, by immunohistochemistry, mHtt was found to be localized as both aggregated as well as diffused forms in the fat body. Targeted expression of mHtt in the fat body resulted in a progressive weight loss of the flies which became more severe with age. For further exploration of alteration in metabolic activity, we monitored the levels of major metabolites including lipids, trehalose, glycogen and protein in these flies. We found significant reduction in lipid levels of these flies and alteration in other metabolites as well. Storage of lipids in the lipid droplets of fat body was also found to be altered suggesting that mHtt

might have deleterious role either in storage or processing of the lipids. Additionally, these flies exhibited reduced viability as compared to age matched controls.

Altogether, our results clearly demonstrate a direct detrimental role of mHtt exon1 peptide with 120Qs on metabolic functioning exclusive of the neurons in an *in vivo* system.

458B Prion-like transmission of mutant huntingtin aggregates in *Drosophila* brains *Margaret Pearce*[1,2], Weizhe Hong[2,3,4], Liqun Luo[2,3], Ron Kopito[2]. 1) Department of Biological Sciences, University of the Sciences, Philadelphia, PA; 2) Department of Biology and Bio-X Program, Stanford University, Stanford, CA, USA; 3) Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA; 4) Departments of Biological Chemistry and Neurobiology, University of California, Los Angeles School of Medicine, Los Angeles, CA, USA.

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG repeat region in exon 1 of the huntingtin (Htt) gene. Htt proteins encoded by this mutant gene contain an expanded polyglutamine (polyQ) stretch near their N-termini and are prone to misfolding and aggregation. Accumulating evidence indicates that mutant Htt protein aggregates have prion-like properties—they spread from one cell to another and convert normally-folded Htt proteins into an aggregated state. We have recently shown in the intact *Drosophila* central nervous system (CNS) that mutant Htt aggregates in olfactory receptor neurons (ORNs) are cleared by neighboring phagocytic glial cells via Draper-dependent phagocytosis. Remarkably, a proportion of these phagocytosed neuronal Htt aggregates reach the glial cytoplasm and effect prion-like conversion of cytoplasmic, wild-type Htt into aggregates. We have also demonstrated that mutant Htt aggregates originating in ORNs can transfer into the cytoplasm of their post-synaptic partners, projection neurons (PNs) and there nucleate aggregation of wild-type Htt. Surprisingly, ORN-to-PN transmission of mutant Htt aggregates also requires Draper, suggesting that glial phagocytosis plays a central role in transferring aggregates between synaptically-connected neurons. Together, these findings demonstrate that pathogenic Htt aggregates can move between individual neuronal and glial cells in intact brains and suggest that phagocytic glia regulate both the clearance and spreading of aggregate neuropathology in the CNS.

459C Drug Screening in *Drosophila* *Tom Hartl*, Tamy Portillo Rodriguez, Ethan Perlstein. Perlara, PBC, San Francisco, CA.

Perlara is a public benefit corporation that discovers small molecule therapies for orphan diseases. Since our conception in 2014, we have focused on the rare diseases Niemann Pick type C (NPC) and N-glycanase 1 (NGLY1) deficiency. *Drosophila* with mutations in these disorders' causal genes, npc1 and ngly1, are developmentally delayed during larval stages. We will present our *Drosophila* high throughput drug screening platform and our progress on discovering small molecule modifiers of the larval developmental delay in these models of human disease.

460A A clinically proven chemical inhibitor rescues Down-syndrome-related phenotypes by inhibition of DYRK1A/minibrain *Kyu-Sun Lee*[1,2], Hyeongki Kim[3], Ae-Kyeong Kim[1], Dae-Woo Kwon[1,2], Eunbyul Yeom[1,2], Kweon Yu[1,2], Seung-Wook Chi[4], Sungchan Cho[3]. 1) Neurophysiology Research Group, KRIBB, Daejeon, South Korea; 2) Department of Functional Genomics, UAS, Daejeon, South Korea; 3) Anticancer Agent Research Center, KRIBB, Daejeon, South Korea; 4) Disease Target Structure Research Center, KRIBB, Daejeon, South Korea.

DYRK1A is important in neuronal development and function, and its excessive activity is considered a significant pathogenic factor in Down syndrome and Alzheimer's disease. Thus, inhibition of DYRK1A has been suggested to be a new strategy to modify the disease. Very few compounds, however, have been reported to act as inhibitors, and their potential clinical uses require further evaluation. Here, we newly identify CX-4945, the safety of which has been already proven in the clinical setting, as a potent inhibitor of DYRK1A that acts in an ATP-competitive manner. The inhibitory potency of CX-4945 on DYRK1A (IC_{50}=6.8 nM) in vitro was higher than that of harmine, INDY or proINDY, which are well-known potent inhibitors of DYRK1A. CX-4945 effectively reverses the aberrant phosphorylation of Tau, amyloid precursor protein (APP) and presenilin 1 (PS1) in mammalian cells. To our surprise, feeding with CX-4945 significantly restored the neurological and phenotypic defects induced by the overexpression of minibrain, an ortholog of human DYRK1A, in the *Drosophila* model. Moreover, oral administration of CX-4945 acutely suppressed Tau hyperphosphorylation in the hippocampus of DYRK1A-overexpressing mice. Our research results demonstrate that CX-4945 is a potent DYRK1A inhibitor and also suggest that it has therapeutic potential for DYRK1A-associated diseases.

461B Drug screening in *Drosophila* *Tamy Portillo Rodriguez*, Tom Hartl, Ethan Perlstein. Perlara, PBC, San Francisco, CA.

Perlara is a public benefit corporation that discovers small molecule therapies for orphan diseases. Since our conception in 2014, we have focused on the rare diseases Niemann Pick type C (NPC) and N-glycanase 1 (NGLY1) deficiency. *Drosophila* with mutations in these disorders' causal genes, npc1 and ngly1, are developmentally delayed

during larval stages. We will present our *Drosophila* high throughput drug screening platform and our progress on discovering small molecule modifiers of the larval developmental delay in these models of human disease.

462C The loop region of Presenilin is essential for Glycogen synthase Kinase-3β mediated axonal transport in *Drosophila* larval axons *Rupkatha Banerjee*, Crystal Naylor, Shermali Gunawardena. Biological Sciences, University at Buffalo,The State University of New York, Buffalo, NY.

Neurons require intracellular transport of essential components for function and viability. Defects in axonal transport have been implicated in many neurodegenerative diseases. Although multiple levels of regulation of motor protein function must exist for proper transport of components within axons, little is known about these mechanisms. One possible mechanism by which transport defects can occur is by improper regulation of molecular motors. Previous work has shown that reduction of Presenilin (PS) or Glycogen synthase kinase-3β (GSK-3β) stimulated APP vesicle motility. Excess GSK-3β causes axonal transport defects and increased motor binding to membranes. Reduction of PS decreased active GSK-3β and motor binding to membranes, together suggesting that PS and GSK-3β may function together during axonal transport. Since PS and GSK3β are known to interact in the β-Catenin pathway, we hypothesize that PS influences GSK3β activity for motor regulation. Using *Drosophila* genetics, we found that excess PS rescued GSK-3β mediated axonal blockages. Intriguingly, the catalytic region of PS (PS loop), which is known to bind to GSK3β, is essential for this rescue. Disruption of PS loop (PSΔE9) exacerbated GSK3β-mediated axonal blocks, while excess of PS loop suppressed it. Together, our observations suggest that functional PS with an intact PS loop region is required to modulate GSK-3β-mediated roles during axonal transport. Perhaps, PS and GSK-3β physically interact to regulate motor activity during axonal transport similar to GSK-3β-mediated mechanisms in the β-Catenin pathway.

463A The Effects of Rapamycin and Alpha-tocopherol in Lethality and Toxicity of *sdhA* Mutants *Rheba R Sam*, Danielle Grushkovskiy, Eugenia Villa-Cuesta. Adelphi University, Biology Department. 1 South Avenue, Garden City.

Debilitating neurological conditions, such as Leigh syndrome, have roots in dysfunction of the electron transport chain of aerobic respiration. Specifically, in *Drosophila melanogaster*, homozygous mutations in the gene which encodes for Subunit A of Succinate-CoQ Oxidoreductase (*SdhA*) are lethal during embryogenesis, and homozygous *SdhA* retina cells obtained by clonal analysis have been shown to contain excess reactive oxygen species. Such retinal cells have exhibited neuronal degeneration of retinal axons, a potential consequence of excessive reactive oxygen species buildup. Rapamycin, an mTOR pathway inhibitor, has been shown to decrease reactive oxygen species levels in the *Drosophila* organismal model (Villa-Cuesta, 2014). This analysis aimed to rescue the lethality of such a homozygous mutation for *sdhA,* by conducting a series of titrations of rapamycin and alpha-tocopherol (a well-known antioxidant) in predetermined concentrations. Our results showed that in response to rapamycin as well as alpha-tocopherol treatments, longevity of homozygous *sdhA* larvae were extended beyond that of untreated *sdhA* mutants. In the pursuit of a potential treatment for severe mitochondrial disorders such as Leigh Syndrome, promising avenues are being currently investigated through the coupling of rapamycin and antioxidant treatments.

464B Rapamycin as a potential treatment for succinate dehydrogenase deficiency *Eugenia Villa-Cuesta*, Frances Fan, Katherine Alvarado, Emma Ryan, Rheba Sam. Department of Biology, Adelphi University, Garden City, NY.

Drosophila melanogaster is a powerful model in understanding the mechanisms underlying mitochondrial respiratory chain defects, particularly succinate dehydrogenase (SDH) deficiency. Mutations in SDH cause degenerative disorders ranging from neurodegeneration to cardiomyopathy and often lead to death. Currently therapies for such pathologies are based on a combination of vitamins and dietary supplements, and are rarely effective. In *Drosophila*, well-characterized mutations in the majority of the SDH subunits produce complications that closely resemble the pathology of SDH deficiency in humans, enabling the *Drosophila* model to be used in finding efficacious treatments for this condition. Here, we investigate the drug rapamycin as a potential treatment for SDH deficiency in *D. melanogaster*. Our results show that exposure to rapamycin improves the survival of individuals with mutant alleles for subunit A (sdhA) and B (sdhB) of SDH, the activity of SDH *in vitro*, and the impaired climbing associated with *sdhB* mutations; however, not all aspects of mitochondrial function benefited from treatment. The production of reactive oxygen species (ROS) in *sdhB* mutants, the oxygen consumption of isolated mitochondria, and the basal metabolic rate were not significantly affected after treatment. The improvement observed in climbing ability was dependent on mitochondrial DNA (mtDNA), and the interaction between mtDNA with SdhB alleles, as some *SdhB* mutant flies with *D. simulans* mtDNA did not exhibit rapamycin-mediated climbing improvement. The results of this research contribute to the current research seeking a treatment for mitochondrial disease in humans.

465C Cell-specific translatomic analysis of injury induced nociceptive sensitization *Kyle Beauchemin*, Geoffrey Beauchemin. Biology, University of New England, Biddeford, ME.

BACKGROUND: Current therapies for the treatment of chronic pain rely heavily on opioids, which are associated with significant risk including deleterious side-effects, dependence, and tolerance. The development of safer and more effective treatments for modulating pain requires a better understanding of the molecular mechanisms associated with injury-induced changes in neuronal sensitivity. Previous studies in *D. melanogaster* have revealed the necessary and sufficient roles of Bone-morphogenetic protein (BMP) and Hedgehog (Hh) signaling in driving sensitization [1]. However, critical questions remain regarding the tissue-specific nature and duration of gene expression changes among downstream effectors involved in producing nociceptive sensitization following injury. Investigating the mediators of this event on a genomic scale has been challenging, however recent sequencing studies and advances in RNA isolation technology have finally made cell-specific quantification of gene expression possible in the fly. With translating ribosomal affinity purification (TRAP) we can now assay the translatome of a specific cell type of interest across biological conditions. To study nociceptive sensitization, we have captured changes among translating RNA in class IV multidendritic neurons (pickpocket-expressing; ppk+) following ultraviolet light induced injury. METHODS: In timed matings between female [ppk-Gal4] and male [UAS-Rpl10Ab-EGFP] flies, young adults were placed on grape juice/agar medium to generate developmentally- synchronized 3rd instar larval progeny expressing GFP-tagged ribosomes only in cells actively expressing the ppk gene. Sensitization was triggered by injuries performed at a consistent time of day using a UV crosslinker set to dispense 14-18mJ of energy. RESULTS: Nociceptor-specific presence of cytoplasmic GFP expression was confirmed by confocal imaging of whole larval filets. Successful immunoprecipitation (IP) of transcripts bound by GFP-tagged ribosomes was confirmed by analyzing the relative expression of neuron- or fat body-specific transcripts between total RNA and IP-RNA. A 34-fold enrichment of ppk expression (nociceptor-specific) and 65-fold depletion of Fbp1 (fat body-specific) was detected in IP-RNA relative to total RNA. DISCUSSION: The goal of our research is to better understand how the normally beneficial and protective process of nociceptive sensitization arises and thereby reveal putative drug targets that represent valuable alternatives to the current pharmacopeia of pain modulatory therapies. REFERENCES: 1. Babcock DT, Shanping S, Juyeon J, Michael S, Gutstein HB, Galko MJ (2011) Hedgehog signaling regulates nociceptive sensitization. Curr Biol 21(18):1525-33.

466A Regulators of BMP signaling control injury induced nociceptive sensitization *Courtney L Brann*, Geoffrey K Ganter. Biology, University of New England, Biddeford, ME.

In the United States alone, over 100 million people suffer from the effects of chronic pain. This burden also impacts the U.S. economy; 600 billion dollars annually is spent on medical care, medications, and lost productivity in the workplace. Current opioid treatments cause adverse effects including nausea, constipation, tolerance, and addiction liability. The neuroplastic process of pain sensitization is thought to perpetuate chronic pain, but very little is known about its mechanisms. Components of the pathways that connect injury and pain sensitization are likely to be valuable targets for novel medications for the relief or prevention of chronic pain. Utilizing the *Drosophila melanogaster* cell targeting and RNA interference toolkit, our lab investigates the Bone Morphogenetic Protein (BMP) pathway and its role in ultraviolet light (UV) injury-induced nociceptive sensitization. BMPs are secreted developmental morphogens that control imaginal disc patterning by binding membrane bound receptors of target cells. We have previously utilized a candidate gene approach to identify BMP signaling components that modulate allodynia in *Drosophila*. The present study investigates the necessity of additional regulators of the BMP pathway in the formation UV injury-induced sensitization. The components of the BMP pathway are highly conserved; in fact they are functionally interchangeable between mammals and flies. Because pain sensitization underlies chronic pain, these genes show potential to represent novel therapeutic targets in humans challenged by chronic pain.

467B Thermal injury results in nociceptive sensitization *Giselle M Dion*, Courtney Brann, Kyle Ryan, Aidan McParland, Geoffrey Ganter. University of New England, Biddeford, ME.

Fruit flies are used to observe the process by which thermal injury results in sensitization of nociceptor neurons. Because pain sensitization perpetuates chronic pain and not much is known about how sensitization works, we have established a model of nociceptive sensitization induced by thermal injury. First, a third-instar larva is placed into a groove cut in an aluminum plate that rests on a tray of ice. Then the larva is covered with a cold water bubble and touched with a probe heated to an injurious temperature. Twenty-four hours later the larvae are tested for sensitization with a warm probe heated to just below the nociceptive threshold (experimental group) or a room temperature probe (control group). Sensitization is indicated if the larva performs a nocifensive roll to this normally innocuous stimulus. The researcher testing for sensitization is blinded so no biases will occur when assessing nocifensive behavior of the larva. Two separate researchers perform the injuring and sensitization testing, as another way of ensuring bias does not occur. Injured larvae can also be dissected for imaging under a confocal microscope. The larvae's dissected body walls are treated with fasciclin antibodies and fluorescently- tagged

secondary antibodies, which makes the edges of the epidermal cells fluoresce and allows for visualization of the damage caused by the thermal injury. The larvae also express Green Fluorescent Protein cell-specifically, which allows for visualization and imaging of the larvae's nociceptor neurons. Results reveal that only larvae burned with a hot probe become sensitized. The larvae treated with a room temperature probe show no sensitization. This could help to better understand through what pathways the fly's nociceptor neurons become sensitized after injury. This knowledge could provide insight into how human patients who have sustained extensive bodily burns become victims of chronic pain even after their injuries have fully recovered and healed. Novel pathways identified using this method of experimentation may represent new targets for medications that will more effectively treat chronic pain.

468C SCF^{Slmb} recognizes a conserved degron within the survival motor neuron (SMN) self-interaction domain to mediate ubiquitylation of SMN and SMNΔ7 isoforms *A. Gregory Matera*, Kelsey Gray, Ying Wen, Amanda Raimer, Ashlyn Spring. Biology, University of North Carolina, Chapel Hill, NC.

Spinal muscular atrophy (SMA) is caused by homozygous loss of human *SMN1 (survival motor neuron 1)*. Expression of a duplicate gene *(SMN2)* primarily results in skipping of exon 7 and production of an unstable protein, called SMNΔ7. Although *SMN2* exon skipping is the principal contributor to SMA severity, mechanisms governing stability of SMN protein isoforms are poorly understood. We used a *Drosophila* model system and 'label-free' proteomics to identify the SCF^{Slmb} ubiquitin E3 ligase complex as a novel SMN binding partner. We show that this interaction is conserved from fly to human, and that SCF^{Slmb} interacts with a phospho-degron embedded within the SMN YG-box self-oligomerization domain. Substitution of a conserved serine (S270A) interferes with SCF^{Slmb} binding and greatly stabilizes SMNΔ7. SMA-causing missense mutations that block multimerization of full-length SMN are also stabilized in the degron mutant background. Furthermore, overexpression of SMNΔ7^{S270A}, but not wild-type SMNΔ7, provides a protective effect in SMA model mice and human motor neuron cell culture systems. Our findings support a model wherein the SCF^{Slmb} degron is largely exposed when SMN is monomeric, whereas it is sequestered when SMN forms higher-order multimers. SMN stability is thus regulated by self-oligomerization, providing an elegant mechanism for controlling functional activity.

469A Abberant planar spindle orientation induces epithelial plasticity in *Drosophila* *Yu-ichiro Nakajima*[1], Christopher Seidel[2], Matthew Gibson[2]. 1) Tohoku University, Sendai, Miyagi, Japan; 2) Stowers Institute for Medical Research, USA.

Polarized epithelia cover the surface of animal bodies and organs and are the primary cellular source of human cancers. During development and homeostasis, epithelial cell division typically occurs by orienting the mitotic spindle to the plane of the epithelium (planar spindle orientation). Although defects in this process have been proposed to contribute to epithelial disease, it is still unclear whether spindle misorientation is a cause or consequence of epithelial cell abnormalities and tissue disorganization *in vivo*. Recently we found that abnormal planar spindle orientation results in basal cell delamination and apoptotic cell death in the *Drosophila* imaginal disc epithelia. Intriguingly, when apoptosis is blocked, daughter cells from misoriented divisions exhibit characteristics of epithelial-to-mesenchymal transition (EMT), including loss of E-cadherin, upregulation of Matrix metalloproteinases, and formation of disorganized cellular masses. In order to determine the genome-wide transcriptional changes associated with delaminating cells, we performed RNA-seq analysis and found upregulation of stress signaling components, cytokines, and EMT-related transcriptional factors, as well as downregulation of genes controlling wing cell identity. Ectopic expression of EMT transcriptional factors mimics EMT-like effects that are observed when abnormal spindle orientation is induced. These findings suggest that aberrant planar spindle orientation triggers EMT-like effects, which is sufficient to initiate a mesenchymal-like transcriptional program and convert cellular identity, and open a new experimental avenue for understanding abnormal epithelial plasticity and pathogenesis.

470B The role of planar cell polarity (PCP) signaling in tumor progression in *Drosophila* *Joy Y. Wan*[1,2], Bomsoo Cho[2], Jeffrey D. Axelrod[2]. 1) Department of Biology, Stanford University, Stanford, CA; 2) Department of Pathology, Stanford University School of Medicine, Stanford, CA.

Planar cell polarity (PCP) signaling governs a multitude of directional cell behaviors, including the tissue-level organization of epithelial cells with coordinated polarity orthogonal to the apico-basal axis. Components of the PCP signaling pathway are well conserved from flies to vertebrates. PCP components have been proposed to play a role in tumor progression in part because they regulate collective cell movement during various morphogenetic events. Although studies have linked PCP to tumor progression, they do not provide a complete picture of how PCP regulates this complex process. We investigated the role of core PCP components Frizzled (Fz), Dishevelled, Diego, Flamingo (Fmi), Van Gogh, and Prickle (Pk) in tumor progression in *Drosophila*. To study whether core PCP components play cell-autonomous roles in early tumor growth, we induced RFP-labeled malignant
tumors overexpressing Ras^{V12} and *scribbled* (*scrib*) RNAi in the entire *Drosophila* eye-antennal disc and mutated or knocked down these components. We found that removing each of the core PCP genes, except *pk*, had no effect on tumor growth. To assess whether core PCP components play cell non-autonomous roles, we generated RFP-

labeled *ras*^{V12} *scrib* RNAi tumor clones, which lacked core PCP genes, in the eye-antennal disc. Intriguingly, we found that removing *fmi* gene function and knocking down *fz,* but not the other core PCP genes, in these tumor clones suppressed their outgrowth. Since these inhibitory effects were seen only in the presence of surrounding non-tumor cells, our results suggest that core PCP components may play a role in cell competition. Our studies, which leverage our understanding of PCP-driven cell-cell communication mechanisms, may give valuable insight into competitive interactions between tumor cells and non-tumor cells to regulate tumor growth.

471C Modulation of autophagy and Nrf2 signaling ameliorate defects caused by cardiac expression of mutant lamins *Shruti Bhide*[1], Sreehari Kalvakuri[2], Sahaana Chandran[1], Maureen O'Connor[3], Grant Young[3], Diane Cryderman[3], Adriana Trujillo[1], Mastaneh Nikravesh[1], Rolf Bodmer[2], Lori Wallrath[3], Girish Melkani[1,2]. 1) Dept. of Biology & Molecular Biology Institute, San Diego State University, San Diego, CA; 2) Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; 3) Dept. of Biochemistry, School of Medicine,University of Iowa,IA.

Laminopathies are a group of genetic disorders caused by dominant mutations in the human *LMNA* gene, which encodes the developmentally regulated A-type lamins. Lamins are intermediate filaments proteins that line the inner nuclear membrane and provide structural support for the nucleus. Patients with laminopathies exhibit a wide range of disorders including cardiac and skeletal muscle dysfunction, dysplasia, diabetes, and progeria. In particular, cardiomyopathy is the major cause of death in laminopathy patients; however the underlying molecular mechanisms are not well understood. We generated Drosophila models to dissect the physiological and pathological roles of lamins in cardiac function. Specifically, we generated flies that express the Drosophila Lamin C gene encoding amino acid substitutions (*LamC R205W* and *G489V)* that are analogous to those associated with cardiomyopathy in humans. Cardiac-specific expression of mutant LamC, but not the wild type, resulted in compromised cardiac function accompanied by cytoplasmic aggregation of lamins, mislocalization of Nrf2, disruption of the cytoskeleton, nuclear blebbing and mitochondrial dysfunction. In addition, expression of mutant *LamC* resulted in elevated levels of triglycerides and a shortened lifespan. We hypothesize that mutant lamin and/or its cytoplasmic mislocalization triggers signaling pathways that alter cellular redox and metabolic homeostasis, resulting in cardiac muscle dysfunction. Using a combination of genetic, physiological, and biochemical analyses, we have shown that over-expression of ATG1 and RNAi knock-down of *Nrf2* resulted in improved cardiac function, reduced cytoplasmic aggregation of lamin and relocalization of Nrf2 to the cytoplasm. Moreover, we have shown that simultaneous over-expression of Atg1 and RNAi knock-down of Nrf2 are required for *complete suppression of the cardiac phenotypes and shortened lifespan.* These findings suggest pathways and targets that are potential avenues for therapy for laminopathy patients.

472A Nascent polypeptide Associated Complex-alpha is required for cardiac morphogenesis, particularly during cardiac remodeling in Drosophila. *Analyne M Schroeder*, Georg Vogler, Alexandre Colas, Rolf Bodmer. Development, Aging and Regeneration, Sanford Burnham Prebys Medical Discovery Institute, La Jolla , CA.

Heart Disease has a significant impact on our society and efforts to identify novel pathogenic genes and to understand the mechanisms attributing to cardiac defects are warranted in order to offer optimized treatments. Using patient-derived genomic information, candidate genes were identified and tested in Drosophila; the knockdown (KD) of the candidate gene Nascent polypeptide Associated Complex-alpha (*Nacα)* throughout development of the fly heart produced adult flies that lacked a heart. KD of *Nacα* strictly in adult hearts did not alter function or structure compared to controls suggesting that *Nacα*, is not necessary for mature cardiomyocyte maintenance, but may play a protein-targeting role particularly during heart morphogenesis. Closer examination of heart pathogenesis during pupal stages suggests that complete heart lysis occurs during metamorphosis, indicating that *Nacα* may interact with genes pertinent to cardiac remodeling. We, therefore, tested for a genetic interaction between *Nacα* and the *hox* gene *abd-B,* a gene critical for proper remodeling to an adult heart by inducing lysis of the most posterior cardiomyoctes of the larval heart. We hypothesized that *Nacα* KD induces ectopic *abd-B* expression throughout the heart resulting in histolysis of the entire heart. Overexpression of *abd-B* in the heart indeed caused loss of the heart in adult flies. The KD of both *Nacα* and *abd-B* genes resulted in partial rescue: a beating heart was present in adults but with compromised structure and function, indicating a genetic interaction. KD of *Nacα* in human multipotent cardiomyocyte progenitor cells disrupted differentiation into cardiomyocytes. Results thus far suggest a role of *Nacα* in heart morphogenesis particularly during metamorphosis, and may regulate protein subcellular localization and expression, including that of *abd-B,* that are necessary for cardiac reprogramming and differentiation. A better understanding of *Nacα*'s function will aid in expanding our knowledge of mechanisms driving cardiac development and pathogenesis that may lead to improved diagnosis, novel therapies and optimized care for heart disease patients.

473B Hypertrophic and Dilated Cardiomyopathy Myosin Mutations Cause Severe Striated Muscle Defects in a *Drosophila* Model *Adriana Trujillo*[1], William Kronert[1], Meera Viswanathan[3], Girish Melkani[1], Anju Melkani[1], Karen Hsu[1], Tom Huxford[2], Anthony Cammarato[3], Sanford Bernstein[1]. 1) Department of Biology and SDSU Heart Institute, San Diego State University, San Diego, CA; 2) Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA; 3) Division of Cardiology, School of Medicine, Johns Hopkins University, Baltimore, MD.

Transgenic lines were generated to express myosin harboring cardiomyopathy mutations to test the hypothesis that these mutations cause hypertrophic (HCM) and dilated (DCM) cardiomyopathy by enhancing or reducing myosin activity, respectively. A human K146N HCM mutation localizes to the N-terminus of β-cardiac myosin heavy chain, and molecular modeling of the analogous *Drosophila* R146 residue predicts its electrostatic interaction with an E774 residue of the lever arm during the pre-power stroke state. R146N myosin displayed increased Ca-ATPase and basal Mg-ATPase levels compared to controls, consistent with the hypothesis that HCM mutations enhance myosin activity. In contrast, R146N myosin showed no change in actin-activated ATPase levels and motility of F-actin was reduced. Cardiac defects in 3 week-old heterozygotes included smaller cardiac diameters with reduced fractional shortening, indicating a restricted cardiomyopathy phenotype, as well as myofibrillar disarray/degeneration. These data suggest that a conserved interaction between the N-terminus and lever arm is critical for myosin function as well as the structure and function of striated muscles. DCM mutations S532P and R369Q are predicted to interfere with actin binding due to their location within known actin binding sites. Transgenic homozygotes expressing S532P and R369Q mutations display progressive disruptions in flight ability, indicating reduced muscle function. To determine the residue interactions disrupted by each HCM or DCM mutation, we expressed and purified His-tagged versions of mutant myosins from transgenic indirect flight muscles by Ni column chromatography. These are being employed for crystallography to define mutant defects at the atomic level. We conclude that HCM and DCM myosin mutations cause striated muscle functional defects. In concordance with other models, HCM myosin ATPase activity is enhanced and we are assessing whether DCM myosin activity is depressed. Overall, we developed an integrative approach to determine the mechanistic basis of contractile dysfunction and pathological heart remodeling due to myosin cardiomyopathy mutations.

474C Neuropeptide F is a Target of Developmental Ethanol Exposure in *Drosophila melanogaster* *Amanda Guevara*, Hillary Gates, Rachel Vasquez, Rachael French. San Jose State University , San Jose , CA.

Fetal Alcohol Spectrum Disorder (FASD) describes a collection of physical and neurobehavioral abnormalities that are a result of developmental alcohol exposure (DAE). Despite decades of research on DAE many of the underlying molecular pathways targeted by ethanol are largely unknown. We use *Drosophila melanogaster* to study FASD and to examine the effects of DAE on the function and expression of the feeding hormone Neuropeptide F (NPF). Using spectrophotometric analysis of dyed food to measure food intake, we find that ethanol-reared flies eat less than control flies, which is consistent with the effects seen in mammals with FASD. In addition, ethanol-reared flies show reduced motivation to eat, as measured by the time that elapses between feeding bouts when flies have free access to food. Furthermore, our data indicates that NPF expression is increased in the brains of ethanol-reared larvae, and that partial loss of function of NPF enhances the feeding deficits seen in ethanol-reared flies. Most strikingly, we find that flies with a null mutation in the NPF receptor (*NPFR1*) display drastically reduced survival when reared in ethanol compared to control animals. This result is unexpected, as there are no published data indicating a survival requirement for NPF signaling in any species. We have found that the critical period for *NPFR1* in ethanol-reared flies is during larval development and these data suggest that increased NPF expression is a protective mechanism against reduced feeding for ethanol-reared flies and we are now investigating the possibility that the animals die of starvation due to insufficient neurochemical motivation to feed.

475A The effect of dietary modifications on the expression of fetal alcohol syndrome in *Drosophila melanogaster* *P. Khouderchah*, R. French. San Jose State University , San Jose, CA.

Fetal alcohol syndrome (FAS) and fetal alcohol spectrum disorder (FASD) are caused by ingestion of alcohol during fetal development. The ability of alcohol to permeate the blood brain barrier leads to particularly detrimental forms of mental retardation, behavioral alterations and stunted physical development. Some deleterious phenotypes presented as a result of developmental alcohol consumption may be treated with medication, but most are not, and there is no drug available for the specific treatment of FAS. Finally, though the detrimental effects of alcohol are widely known, there are still 20,000 to 200,000 diagnosed cases of FAS in the United States per year.

We have established a *Drosophila* model for developmental ethanol exposure (DAE). Using this model, we can study the molecular effects of DAE, with the ultimate goal of developing treatments. We have generated mutations that alter survival and development time in flies reared in ethanol. Among these, we identified three DAE-sensitive alleles of *whd*, which encodes the fly homolog of carnitine palmitoyltransferase 1 (CPT1). CPT1 is necessary for the shunting of long chain fatty acids into the mitochondrial matrix for beta-oxidation, and its mutation causes disruption of fatty acid metabolism and sensitivity to oxidative stress. We have shown that DAE disrupts insulin signaling and

fatty acid metabolism (McClure et al., 2010, Logan-Garbisch et al., 2014), and that the increased fat storage is one of the causes of the oxidative stress observed as a result of DAE.

Here we connect fatty acid metabolism, oxidative stress, and insulin signaling/sugar metabolism by altering the flies' diet. We show that both low-fat or low-sugar diets lead to resistance to the toxic effects of DAE. In addition, we show that a diet high in long chain fatty acids causes a significant increase in lethality associated with DAE. We are testing the effects of sugar and fat content modifications on the diets of mutants with altered insulin signaling. In addition, we are testing the effects of altered insulin signaling on *whd* mutants, to test the interaction between fatty acid metabolism, insulin signaling, and ethanol-induced developmental toxicity.

476B Identification of rare disease patients with variants in *TBX2*, *TBX3*, and molecular functional studies by using *Drosophila* *N. liu*[1], L. Pena[2], C. Toro[3], K. Schoch[2], W. Gahl[3], S. Yamamoto[1,4,5], M. Wangler[1,4,5], M. Malicdan[3], V. Shashi[2], H. Bellen[1,4,5,6]. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030; 2) Division of Medical Genetics, Department of Pediatrics, Duke Health, Durham, NC 27710, USA; 3) NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH and National Human Genome Research Institute, NIH, Bethesda, MD, 20892, USA; 4) Jan and Dan Neurological Research Institute, Texas Children's Hospital, Houston, TX, 77030, USA; 5) Program in Developmental Biology, Baylor College of Medicine, Houston, TX, 77030, USA; 6) Howard Hughes Medical Institute, Baylor College of Medicine, Houston TX 77030, USA.

Whole-Exome Sequencing (WES) and Whole-Genome Sequencing (WGS) provides powerful clinical diagnostic tools to identify possible novel disease causing variants from patients with undiagnosed rare diseases. The Undiagnosed Diseases Network (UDN) aims to bring together clinical and model organism (fruit flies and zebrafish) experts to determine pathogenicity of candidate variants by providing phenotypic information. As part of UDN team, the Model Organism Screening Center at BCM is assessing the function of variants of genes *in vivo* specifically variants in *TBX2* and *TBX3*. A missense variant in the *TBX2* gene co-segregates in an autosomal dominant inheritance pattern with the phenotype of absent thymus, cleft lip and palate, heart defects and bone anomalies. These phenotype overlaps with the findings of DiGeorge syndrome, linked to *TBX1*. A homozygous missense allele in *TBX3* appears related to an adult phenotype with altered immunoglobulins, polyneuropathy, chronic pain, cardiomyopathy and cognitive impairments, which would constitute a phenotypic expansion for this gene. These two vertebrate genes are the only obvious homologs to the *Drosophila bifid/omb* gene, a transcription factor with repressor activity which plays a critical role during the development of optic lobes, wings and abdominal pigmentation. A P-element insertion in the *omb* gene expresses the Gal4 in the proper spatial and temporal pattern and this allele corresponds to a weak partial loss of function mutation. However, a strong wing phenotype is observed when this allele is tested over a severe loss of function mutation. This phenotype provides for an assay to test the human genes in flies. Transgenic flies were created that carry the *UAS-human TBX2 and TBX3 cDNA* and the mutant variants and rescue of the wing phenotype is currently being assessed.

477C Establishing *Drosophila melanogaster* as a model for the identification of environmental chemicals that confer risk of autism in genetically susceptible individuals *K. Nguyen*, L. Murphy, B. Trafton, H. Sidhu, K. Ghenta, A. Lucich, R. Corona, K. Ghuman, A. Lopez, C. Torres, Z. Hindi, T. Doan, K. Mulligan. California State University, Sacramento, Sacramento, CA.

Mounting evidence indicates that the interaction of environmental chemicals with specific genetic susceptibilities is linked to autism spectrum disorder (ASD), a condition that afflicts 1 of every 68 children in the United States. Given that 2,000 new chemicals are introduced each year with little to no toxicological data, the field desperately needs an efficient method for screening chemicals to identify those that confer risk to ASD. This project involves the development of assays using *Drosophila melanogaster* for the rapid identification of chemicals that molecularly converge with *fmr1* (*fragile X mental retardation 1*). In humans, null mutations in *fmr1* cause fragile X syndrome and are the most prevalent single gene cause of ASD. *Fmr1* is conserved from flies to humans and *fmr1 Drosophila* exhibit neurodevelopmental phenotypes consistent with vertebrate model organisms. Studies suggest that gestational exposure to environmental chemicals, like polychlorinated biphenyl 95 (PCB-95), can significantly increase the severity of *fmr1* mutant phenotypes in mammals. METHODS & RESULTS: We exposed embryonic and larval stage *wild-type* (*wt*) and *fmr1 Drosophila* to PCB-95. The courtship assay, a well-established paradigm for behavioral analysis of *Drosophila*, was used to show that developmental exposure to nanomolar concentrations of PCB-95 significantly decreases the courtship index (CI; a quantitative measure of courtship behaviors) of both *wt* and *fmr1* mutant flies, reflecting the ability of PCB-95 to impair the neurodevelopmental program. Notably, PCB-95 exposure lowers the CI of *fmr1* flies more significantly than *wt* flies, indicating that null *fmr1* mutations increase susceptibility to PCB-95 in *Drosophila*. This result is consistent with findings in vertebrate model organisms and supports the use of *Drosophila* for chemical screening to identify those that enhance *fmr1*-associated impairments. To complement behavioral analysis, we examined axon-pathfinding defects in an adult neural structure called the mushroom body (MB). We found that PCB-95 exposure during development increases b-lobe axon pathfinding defects at the midline of MBs in *wt* flies. Strikingly, this specific defect is known to occur in *fmr1* mutant

flies, indicating a shared cellular mechanism of action by *fmr1* and PCB-95. We are currently investigating if the severity of this defect is enhanced by PCB-95 in *fmr1* mutant flies.

478A Genetic and environmental modifiers of lethality in a *Drosophila* model of *NGLY1* deficiency indicate potential therapeutic approaches *Katie G. Owings*, Clement Y. Chow. University of Utah, Salt Lake City, UT.

NGLY1 deficiency, the only known human disease of deglycosylation, is caused by autosomal recessive loss-of-function mutations in *N-Glycanase 1* (*NGLY1*). Patients with *NGLY1* deficiency present with developmental delay, movement disorder, seizures, hypotonia, liver dysfunction, and alacrima. NGLY1 is a conserved component of the endoplasmic reticulum associated degradation (ERAD) pathway. ERAD is responsible for degrading misfolded proteins that accumulate in the lumen of the ER. NGLY1 deglycosylates misfolded proteins in the cytoplasm as they are translocated from the ER lumen for degradation. We used ubiquitous RNAi knockdown (KD) of *Pngl* (*Drosophila* ortholog of *NGLY1*) to model complete loss of NGLY1 activity observed in human patients. RNAseq analysis of *Pngl* KD flies revealed a deficiency in GlcNAc synthesis. We hypothesized that restoring the levels of UDP-GlcNAc in *Pngl* KD flies would rescue some of the lethality associated with *NGLY1* deficiency. We show that supplementing our normal *Drosophila* diet with GlcNAc can rescue developmental lethality associated with loss of *Pngl* activity. When raised on normal food, without GlcNAc supplementation, we observed significant lethality, with eclosion of only 18% of expected *Pngl* KD adults. When diet is supplemented with GlcNAc, we observed significant rescue of the developmental lethality, raising the adult *Pngl* KD eclosion rate to nearly 70% of expected. RNAseq analysis also revealed an upregulation of the heat shock response in *Pngl* KD flies. We demonstrate that inducing a stronger heat shock response through environmental and genetic modification of the cytoplasmic heat shock pathway rescues the developmental lethality of *Pngl* RNAi flies. Finally, through a natural genetic modifier screen using the *Drosophila* Genetic Reference Panel (DGRP), we provide evidence that *Sel1L* variants modify lethality of *Pngl* deficient flies. These data suggest a plausible pathophysiology for *NGLY1* deficiency. Importantly, these different methods of rescue indicate different mechanisms that might be exploited for the development of therapeutic approaches to treat *NGLY1* deficiency.

479B Regulation of Dpp release by Irk2 is required for proper *Drosophila* wing development *S Pradhan*[1], G Dahal[1], R Moreno[2], A Ribera[2], E Bates[1]. 1) Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO; 2) Department of Physiology, University of Colorado Anschutz Medical Campus, Aurora, CO.

Disruption of the Kir 2.1 inwardly rectifying potassium channel causes craniofacial and limb abnormalities. In *Drosophila*, disruption of a Kir2.1 homolog (Irk2) causes developmental defects in wings and in eyes by affecting the bone morphogenetic protein (BMP/Dpp) pathway. Our goal is to understand how a potassium channel contributes to Dpp signaling. Inwardly rectifying K^+ channels set the resting membrane potential and therefore regulate neuro-peptide release in neurons and insulin release in pancreatic beta cells by modulating electrical activity and calcium levels. We hypothesized that Irk channels may regulate Dpp release by modulating membrane potential. Here we show that Irk channels are required for pulsatile release of Dpp-GFP from Dpp producing cells and that this affects downstream signaling. Inhibition of Irk channels affects innate electrical and calcium activity. Electron microscopy shows that Dpp is stored in vesicles. Live imaging shows that Dpp release can be evoked with high potassium solution depolarization, as in excitable cells. Together, our data show that in *Drosophila* wing discs, inhibition of an ion channel alters native electrical activity, Dpp signaling in the wing disc, and ultimately morphogenesis of the wing.

480C O-glycosylation is required for peritrophic membrane formation to maintain gut homeostasis in *Drosophila* *Liping Zhang*, Kelly Ten Hagen. NIDCR/NIH, Bethesda, MD.

The mucous layer is important for the luminal protection of the mammalian gastrointestinal tract. It is composed of large, highly glycosylated proteins known as mucins, which are secreted by goblet cells. Defects in the formation of the mucous layer are associated with human intestinal diseases, such as colitis and colon cancer. In *Drosophila,* there is the similar mucous layer known as the peritrophic membrane (PM), which forms along the intestinal tract and serves as a barrier to microbial infection as well as a lubricant for food passage. Because of the high degree of conservation between *Drosophila* and mammals with respect to signaling pathways that control intestinal development, *Drosophila* has been used to gain insights into the factors involved in intestinal regeneration and disease. Here, we employ *Drosophila* to study the function of the mucous layer in intestinal homeostasis and regeneration. We find that the loss of an O-glycosyltransferase (PGANT4) disrupts formation of the PM. PGANT4, which regulates polarized secretion in secretory cells of the digestive tract, is required for proper PM formation. Loss of PGANT4 resulted in the disruption of the PM along the entire digestive tract. Moreover, loss of PM results in activation of the immune response, epithelial cell detachment and apoptosis. Our findings demonstrate the importance of O-glycosylation and the mucous membrane in intestinal protection and homeostasis, and provide a good model for studying how disruptions in this membrane lead to intestinal pathology and inflammatory diseases.

481A Exploring the molecular mechanism of *Drosophila*Thin/dTRIM32 implicated in the pathogenesis of Limb Girdle Muscular Dystrophy 2H*.* *SIMRANJOT BAWA*, ERIKA GEISBRECHT. BIOCHEMISTRY AND MOLECULAR BIOPHYSICS, KANSAS STATE UNIVERSITY, MANHATTAN, KS.

The E3 ubiquitin ligase Trim32 is a member of tripartite motif (Trim) family of proteins involved in various processes including differentiation, cell growth, muscle regeneration and cancer. Trim32 is conserved between vertebrates (humans, mouse) and invertebrates (*Drosophila*). The N-terminus of this protein is characterized by a RING domain, B-box domain, and coiled coil region, while the C-terminus contains six NHL repeats. In humans, mutations that cluster in the NHL domains of TRIM32 result in muscle disorders ; Limb Girdle Muscular Dystrophy type 2H (LGMD2H) and Sarcotubular Myopathy (STM). Mutations in the B-box region cause Bardet-Biedl Syndrome (BBS), a clinically separate disorder that affects multiple parts of the body. A comprehensive genetic analysis in vertebrate models is complicated by the ubiquitous expression of TRIM32 and neurogenic defects in *TRIM32-/-* mutant mice that are independent of the muscle pathology associated with LGMD2H. The model organism *Drosophila melanogaster* possesses a Trim32 [dTrim32/Thin (Tn)/Abba] homolog highly expressed in muscle tissue. We previously showed that dTRIM32 is localized to Z disk of the sarcomere and is required for myofibril stability. Muscle form correctly in *Drosophila tn* mutants, but exhibit a degenerative muscle phenotype once contraction ensures. Mutant or RNAi knockdown larvae are also defective in locomotion, which mimics clinical features associated with loss of TRIM32 in LGMD2H patients. It is predicted that mutations in the NHL domains either affect protein structure or are involved in protein-protein interactions. However, the molecular mechanism by which these mutations affect the interaction properties of dTRIM32 is not understood. Biochemical pulldown assays using the bait fusion protein GST-dTRIM-NHL identified numerous dTrim32 binding proteins in larval muscle tissue. Many key glycolytic enzymes were present in the dTrim32 pulldowns and not in control experiments. Glycolytic genes are expressed in the developing *Drosophila* musculature and are required for myoblast fusion. Strikingly, many glycolytic proteins are also found at the Z disk, consistent with Trim32 localization. Our studies are aimed at examining if dTrim32 regulates glycolytic enzyme levels and/or localization to their sites of action.

482B Genetic Basis and Amelioration of Mutant Lamin-Induced Progressive Skeletal Muscle Dysfunction *Sahaana Chandran*[1], Jennifer Suggs[1], Shruti Bhide[1], Sanford Bernstein[1], Steven Moore[2], Lori Wallrath[3], Satchidananda Panda[4], Girish Melkani[1,4]. 1) Dept. of Biology & Molecular Biology Institute, San Diego State University, San Diego, CA; 2) Dept. of Pathology, School of Medicine, University of Iowa; 3) Dept. of Biochemistry, School of Medicine, University of Iowa; 4) Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla.

Laminopathies are genetic disorders caused by dominant mutations in the human *LMNA* gene encoding A-type lamins, which are intermediate filament proteins that line the inner nuclear membrane, providing structural support for the nucleus and regulating gene expression. The goal of this study is gain mechanistic insights and design therapies for lamin-based skeletal muscle diseases. The pathogenic mechanisms of skeletal muscle laminopathies are elusive; a greater understanding is needed for therapeutic interventions. We established *Drosophila melanogaster* models to investigate the mechanistic basis of skeletal muscle dysfunction caused by mutant lamins. In this model, human disease mutations are made in Drosophila *Lamin C* (analogous to *LMNA* mutations that cause skeletal myopathy in humans). Muscle-specific expression of mutant LamC results in a held-up wing phenotype (diagnostic of myofibrillar defects), accompanied by cytoplasmic aggregation of lamins, myofibrillar degradation, upregulation of p62, and impairment of cellular redox homeostasis. We hypothesize that cytoplasmic lamin aggregation triggers signaling pathways that alter cellular redox and metabolic homeostasis, resulting in muscle dysfunction. In support of our hypothesis and to reveal relevance to human pathology, transcriptomics data obtained from patients' muscle biopsy tissues showed alterations of these signaling pathways. We discovered that activation of *AMPK* (down-regulated in patients' biopsies) and RNAi knock-down on *Nrf2* (mislocalized in muscle of laminopathy patients), suppressed mutant lamin-induced muscle laminopathy. In addition, overexpression of PGC1α (an AMPK downstream target) suppressed muscle defects. Our findings provide potential therapeutic targets for lamin-associated skeletal myopathies and other laminopathies such as dilated cardiomyopathy, lipodystrophy and Hutchison-Gilford progeria syndrome.

483C Stress and DGC collaboratively regulate muscle dystrophy pathways *Ibrahim Omer Cicek*, Halyna Shcherbata. Gene Expression and Signaling, Max Planck Institute, Goettingen, Germany.

Muscular dystrophies are lethal hereditary neuromuscular conditions, which are associated with dystrophinglycoprotein complex (DGC) deficiencies. The fact that the members of the DGC are evolutionarily well conserved makes *Drosophila melanogaster* a great model to study DGC function and the nature of muscular dystrophies caused by deficiencies in DGC. We have previously found that different types of stress accelerate the muscular dystrophy onset in the dystrophic fly model and are able to induce acute muscle degeneration in wild type flies. In order to identify the cellular mechanisms, which are governed by the DGC and stress response pathways, we generated genome-wide transcriptome data. We performed an RNASeq experiment by using different mutants of

DGC units: Dystroglycan (Dg), Dystrophin (Dys), Syntrophin-like 1 (Syn1), and Nitric oxide synthase (Nos). In addition, we used different nutritional and temperature stress conditions in order to reveal affected cellular processes. We identified numerous pathways to be DGC and/or stress dependent. Our data provides insight how the stress response mechanisms and DGC can collaboratively govern gene expression and control signaling pathway networks. To further dissect the precise involvement of the DGC in the gene expression changes, we focused on one of the downstream effectors of the DGC and stress response pathways, the microRNAs (miRNAs). miRNAs are short non-coding RNA molecules, which regulate the expression of their target genes posttranscriptionally. They base-pair with the 3'UTR of the target mRNA and modulate its stability and/or access to the translational machinery. As stress response factors, miRNAs are robust regulators of gene expression, where they ensure a quick and strong response in the target gene expression levels without the need of a transcriptional control. We have assayed the changes in miRNA expression profiles in mutants of DGC elements and in wild type flies under stress conditions. Now, we are compiling these two data sets to establish and confirm the complex DGC-miRNA-stress network, which underlies the cross-connected pathway of the muscular dystrophy and stress. Using our dystrophic fly model and transcriptomics we hope to shed light on the cellular mechanisms of muscular dystrophies and provide context for the search for possible treatments.

484A ***Locomotor activity of dystrophy mutant larvae of Drosophila melanogaster***

Nataliia Holub, Kopij Oksana, Olijnyk Solomiia. Genetics and Biotechnology, Ivan Franko National University, Lviv, Lvivska, Ukraine.

Dystrophin is one of the best characterized protein of dystrophin-dystroglycan complex (DGC). Its absence in humans causes development of Duchenne muscular dystrophy (DMD) which is characterized by a destabilization of the DGC, sarcolemmal fragility, myofiber degeneration and muscle weakness. It’s X-linked recessive disease starts at the age of 4-5 years and affects boys mainly. Persons with DMD live up 25– 30 years. *Drosophila melanogaster* is a good model organism for investigating the problems of DMD. In our previous experiments we investigated behavioral assays of *Drosophila melanogaster* dystrophy adults. It was shown that in dystrophy mutants some indexes were reduced. We decided to analyze the behavioral reactions of dystrophy mutant larvae of *Drosophila melanogaster.* . In every test 300 early third instar larvae (72–78 h) of strains *Oregon* (wild type) and *DysDf//DysDf* (with deletion of dystrophy gene) were used.

In our study we analyzed locomotor activity, foraging test and intensity of contraction of the larvae body.

The index of locomotor activity of dystrophy mutants was 2,88 cm in a minute in average. In wild type larvae it was higher – 3,78 cm in a minute. It can evidence that the mutant dystrophy signs of muscle damage manifest in larval stage. A $_{t1/2}$ arrival time, or time at which half of the total number of larvae in assay reached the yeast (food) was determined. In dystrophy larvae this time was 3,79 minutes. The wild type larvae moved more quickly and reached the yeast in 1,73 minutes. In *DysDf//DysDf* larvae an average index of body contractions amounted to 18,15 times in a minute while in *Oregon* larvae it was lower – 15,36 times.

Also we investigated total egg-laying and larvae amount in strain *Oregon* and heterozygote dystrophy mutants *DysDf//TM6Tb*. According to the results in wild type strain the total number of deferred eggs was 6119 and 4023 larvae hatched from them. In dystrophy mutants these indexes were lower and amounted to 4471 and 1498 respectively.

So it was shown that in dystrophy larvae the index of locomotor activity was statistically lower comparing to *Oregon* larvae but an arrival time and amount of body contractions were higher. In wild type strain the number of larvae that hatched was 66% while in dystrophy mutants the total egg-laying was statistically lower and number of hatched larvae amounted to 34%.

485B **Effects of Protein Expression Manipulation on Muscle Structure and Function in Inclusion Body Myopathy-3**

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Inclusion body myopathy type 3 (IBM-3) is a rare, dominant disease characterized by progressive degradation of skeletal muscle in patients expressing an E706K substitution in myosin heavy chain type IIa. We created a *Drosophila* IBM-3 model by expressing the analogous mutation, E701K, in indirect flight muscle (IFM). Homozygotes display thermally unstable myosin, progressive myofibrillar degradation, and myofibers with autophagic vesicles and membranous inclusions. To identify aggregated proteins within inclusions, we extracted insoluble proteins from IFM of young and old IBM-3 homozygotes and age-matched controls. Quantitative iTRAQ proteomic comparison revealed 18 proteins with >1.5-fold difference in relative abundance at both ages of mutant IFM vs. controls. Two of six proteins over-represented in IBM-3 had small heat shock protein domains (Hsp20, CG7409). Twelve under-represented proteins included Hsp23, Hsp60, and Trim32 (abba/thin), which has ubiquitin-protein transferase activity. Other proteins have unknown functions or play structural, metabolic, signaling, synthesis, or transcriptional roles. We hypothesize that a) these proteins are critical for wild-type (WT) muscle development and function and b) altering their expression will affect the IBM-3 heterozygote (E701K/+) phenotype. To test this, we are

using the GAL4-UAS system to modulate expression of the identified genes in WT and E701K/+ IFM and assessing flight ability. IFM-specific driver lines Act88F-GAL4 and fln-GAL4 were crossed with UAS-RNAi lines to knock-down each gene. Flight was impaired in young WT Act88F-GAL4> [oxen] or [Trim32] gene knock-down. Further, over-expression of CG4463 (Hsp23) decreased flight ability. Our data indicate that levels of Trim32, oxen, and Hsp23 are important for muscle function. We now are assessing structural changes in the wild-type muscle and examining effects in E701K/+ mutants. Overall, these studies will lead to an understanding of the roles that specific aggregation-prone proteins play in normal and diseased muscle structure and function.

486C Genetic Variation for Diabetic-like Traits in the Fly Disease Model *Xuan Zhuang*[1], Gabriella Ceresa[1], Michael Ludwig[1], Soo Young Park[2], Graeme Bell[2], Martin Kreitman[1]. 1) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL.

Type 2 diabetes (T2D) results from a complex interplay between genes and environment. Genome-wide association studies (GWAS) have identified many associated loci for T2D, though these loci account for less than 10% of the heritable component of the disease. Drosophila is well established as a model for investigating natural variation for quantitative traits, and it provides exceptionally powerful tools to dissect the relative contributions of both genes and environment on metabolism. Glucose metabolism is regulated by highly conserved insulin signaling pathways. Previous studies revealed a high sugar diet has profound effects on cellular physiology and metabolism in Drosophila, including insulin resistance, lipid metabolism, impaired lifespan and reduced body size. Here, we developed a fly disease model with diabetic-like traits induced by carbohydrate-rich diet. We reared flies on high and low sugar diets (HSD and LSD) and examined whole body glucose, glycogen, triglyceride levels and body weight in different stages of their life cycle, along with developmental rate, survivorship, and longevity. We seek to survey natural genetic variation for these traits across a subset of Drosophila Genetic Resource Panel (DGRP) lines, and conduct GWAS to identify candidate genes by focusing our association tests on homologs of human disease genes. On average the HSD fed flies are associated with an increase in glucose level, a decrease in body weight, developmental rate, survivorship, and longevity, comparing with the LSD fed flies. We found significant genetic variation for these phenotypes across the examined lines and several correlations between these phenotypes. In addition, we also develop a unique resource for mapping natural variation in Drosophila, consisting of 64 DGRP lines, which have been combined into 16 highly recombinant synthetic cage populations, each of which is founded by 8 of the inbred lines. Fly embryos collected from these synthetic populations are reared on HSD and LSD, and then the flies with extreme phenotypes of developmental rate are sequenced. Creating bulk segregants between 8 founders instead of 2 provides a greater opportunity for rare variants to be present in the synthetic population and for common variants to be represented, allowing mapping of both rare and common variants. Simulation has shown that this approach has statistical power to identify causative quantitative trait loci (QTLs) not detectable with GWAS alone.

487A RNA-Sequencing of *Drosophila melanogaster* Head Tissue on Various Obesogenic Diets *Wayne Hemphill*, Osvaldo Rivera, Silvienne Sint Jago, Matthew Talbert. University of Louisiana at Monroe, 700 University Ave Monroe, LA 71209.

Approximately 60% of Americans are considered overweight, and 30% are considered obese according to Body Mass Index. Obesity has been shown to increase risk for cardiovascular disease and type-2 diabetes, though it does not guarantee this comorbidity. In addition, it has been implicated in aggravation of neurological conditions such as Alzheimer's. It is critical to understand the molecular mechanisms by which obesity causes comorbidity given its impact on humans. In the model organism *Drosophila melanogaster*, a physiological state mimicking obesity can be induced by subjecting the fruit flies to a medium higher in sugar (HSD) or fat (HFD) than protein. These flies exhibit increased circulating glucose levels, increased triglyceride levels, Dilp2 (insulin) resistance, and behavior indicative of neurological decline, such as decreased climbing ability. Previously, we have assayed the neurological effects of a HFD on flies using associated behavioral-response tests, as well as the transcriptome of the heads of these flies using a microarray followed by qRT-PCR. With this experiment, we are subjecting wild-type flies to both a HSD and HFD, as well as a normal diet to act as our control, followed by an RNA extraction on fly heads of each diet for the purposes of Poly-A selected RNA-Sequencing. This is a novel experiment, as RNA-sequencing analysis of fly heads, which contain a pericerebral fat body, glia, and the brain, and thus all the components of central energy homeostasis, could reveal novel transcriptional patterns of interest. Comparative transcriptome analysis across all three diet types has likewise not been done. RNA-sequencing will be advantageous to us over the microarray and qRT-PCR used in previous experiments, as it provides increased sensitivity to low abundance transcripts, no probe bias, and has the ability to detect transcript variants of interest. If a correlation is suggested between diet and varying transcript levels in neural tissue, this might imply similar dietary effects in a range of other organisms, including humans on an obesogenic diet. We will be targeting up to 65 million paired end reads of 75 basepairs in size utilizing a NextSeq 500 (Illumina), and will be analyzing 4 replicates per dietary condition.

488B Gently induced exercise in adult *Drosophila* exhibits genetic- and sex-specific interactions with larval diet *Kelsey E. Lowman*, Brelahn J. Wyatt, Laura K. Reed. Dept. of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Childhood obesity is a growing epidemic in westernized cultures that leads to lifelong adverse effects, even when corrective measures are taken later in life. Metabolic syndrome (MetS) research is centered on understanding the negative effects of obesity, such as increased risk of heart disease, diabetes, and stroke. Some of the underlying causes of MetS include excess calorie consumption, physical inactivity, sex, and genetic factors. Our research aims to model these factors, their interactions, and their subsequent consequences. In particular, we focus on the negative effects of eating a high-fat diet in the juvenile stages of life and the potential to ameliorate these effects with exercise in adulthood. Our previous work demonstrated that exercise substantially improves adult fly metabolic health in a sex- and genotype-specific manner. In this study, we explore further the effects exercise can have on adult fly health by comparing the outcomes for ten genetically distinct lines from the *Drosophila* Genetics Reference Panel (DGRP) fed a 1.5% high-fat or standard lab diet as larvae that then experienced an induced-exercise verses control treatment as adults. The daily exercise regime followed a five-day inverse pyramid protocol of alternating bouts of exercise and rest within a two-hour window. We performed a negative-geotaxis climbing assay and measured both wet and dry weights. We found that climbing performance and adult weights showed interaction effects among sex, diet, exercise, and genotype. In future work, we will explore the impact of exercise on the underlying physiology, gene expression, and metabolome.

489C The Effects of a High Fat Diet on Memory, Olfaction and Transcriptional Response of the Head in *Drosophila melanogaster.* *Osvaldo Rivera*, Lara Crawford, Silvienne Sint Jago, Matthew Talbert. Natural Sciences, University of Louisiana at Monroe, Monroe, LA.

Obesity predisposes humans to a range of life-threatening comorbidities, including type 2 diabetes and cardiovascular disease. Obesity and its associated dietary habits also appear to aggravate neural pathologies, such as Alzheimer's disease, but this class of comorbidity is less understood. When *Drosophila melanogaster* (flies) are exposed to a high fat diet (HFD) by supplementing a standard cornmeal-sucrose-yeast medium with coconut oil, a rich source of saturated fat, they adopt a now established obese phenotype that results in decreased lifespan, increased triglyceride storage, insulin-like peptide resistance, and hindered climbing ability. The latter of these developments is an indicator of neurological decline in fruit flies. Our objective was to establish the obesity-like phenotype in *Drosophila* and determine a correlation, if any, between obesity and neurological decline in flies through behavioral and transcriptomic analysis. We found that synchronously mated female w^{1118} flies exposed to HFD, maintained an obese phenotype throughout adult life with detectable onset as soon as seven days, evidenced by increased triglyceride stores, diminished life span, and impeded climbing ability. Analysis of gene expression of the fly head via microarray and qRT-PCR validation revealed various functionally and phenotypically relevant genes with significant fold changes. These genes had a range of functions including in memory, metabolism, olfaction, mitosis, cell signaling, and motor function. An Aversive Phototaxis Suppression assay revealed short term memory impairment as a result of (HFD) while no significant difference was seen in odor-seeking ability between conditions. Overall, our initial results point to the suitability of *Drosophila melanogaster* to investigate connections between diet-induced obesity and nervous or neurobehavioral pathology, and to the existence of such a dynamic in a broader range of organisms.

490A SOCS36E, Neurobehavioral Traits and Lifespan, in the *Drosophila Melanogaster* High Sugar Obesity-like State *Silvienne Sint Jago*, Wayne Hemphill, Osvaldo Rivera, Mathew Talbert. Biology, University of Louisiana at Monroe, Monroe, LA.

Obesity is characterized by excess body mass, and an increased risk of pathologies such as diabetes, cardiovascular disease, and neurological decline. A physiological state mimicking mammalian obesity can be induced in *Drosophila melanogaster* by subjecting flies to a solid yeast and sucrose medium that is disproportionately high in sugar content relative to protein content. Flies exposed to obesogenic medium purportedly have increased triglyceride levels, increased circulating glucose, reduced Dilp2 response despite increased expression, cardiac abnormalities, reduced lifespan, and impeded climbing ability. In humans, insulin and leptin resistance results in decreased blood glucose uptake and appetite suppression, and abnormal energy homeostasis, contributing to many of the pathologies associated with obesity. This resistance partly results from an inflammation-upregulated pathway involving the protein suppressor of cytokine signaling 3 (SOCS3), which inhibits leptin and insulin signaling via negative feedback. In *Drosophila,* leptin functional orthologue unpaired 2 (Upd2) is released from the fat body in proportion to nutrient content. Upd2 binds its receptor Domeless in the brain and promotes drosophila insulin-like peptide 2 (Dilp2) release from the median neurosecretory cells ultimately via Hop-Stat92e signaling. The importance of negative feedback inhibitors of Hop-Stat92e signaling in *Drosophila* obesity is unknown. In this study, we reduced adult neuronal expression of SOCS36E (inhibitor of Hop-Stat92e signaling) via the GAL4-UAS system, and

hypothesized that it would result in increased Upd2 effectiveness and Dilp2 responsiveness, thus improving various indicators of obesity pathology. Our objective was to gauge lifespan and neurological health using assays such as lifespan study, negative geotaxis, and aversive phototaxic suppression (APS) to gauge short-term memory. Our negative geotaxis results showed no statistically significant difference in climbing ability between normal and knockdown flies within each diet, or between the diets within each genotype. The APS, however, revealed a statistically significant decrease in retention of trained behavior in normal versus high sugar media with wild-type flies, but no significant difference between the knockdown flies on each media. We will also present the lifespan results, as well as data regarding triglyceride and hemolymph carbohydrate content, of both diets and genotypes.

491B The *Drosophila* TNFα ortholog Eiger regulates tissue tension and cell shape changes through the Crumbs complex to facilitate macrophage tissue invasion Aparna Ratheesh, Jana Vesela, Julia Biebl, Attila Gyoergy, Michael Smutny, *Daria Siekhaus*. IST Austria, Klosterneuburg, Austria.

Immune function and cancer metastasis depend on the ability of cells to move through the tissue barrier of the vascular wall to reach surrounding tissues, in a process called transmigration. TNFα is known to be an important mediator of inflammation and immune cell movement through the vascular wall, yet how it regulates the cellular and mechanical responses of vascular endothelial cells *in vivo* during this process is not clear. We have previously shown that the migration of Drosophila macrophages (plasmatocytes) into the germband during development displays molecular and cellular similarities with vertebrate immune cell transmigration, and we have now used this system to understand the role of Eiger, the *Drosophila* ortholog of TNFα. In fixed embryos, we find that Eiger is required for macrophage invasive migration into the germband, but not for movement along the ventral nerve cord, a step not requiring tissue penetration. 2-photon movies of *eiger* mutant embryos show no alteration in the speed of macrophage migration up to the germband, but do reveal a strong increase in the time macrophages take to move into the tissue. Eiger is known to induce apoptosis upon overexpression through JNK signaling, yet we find no role for either process in this step. Instead we show through laser cuts that Eiger reduces the tension of the ectoderm that flanks the macrophage route of entry into the germband. We demonstrate that Eiger acts through the TNFR ortholog Grindelwald and members of the Crumbs complex. Depletion of Eiger results in reduced apical ectodermal localization of Patj, a Crumbs complex component that binds Myosin Phosphatase; this leads in *eiger* mutants to higher apical levels of phosphorylated Myosin (*sqh*) and tissue tension. The reduced entry of macrophages in *eiger* mutants can be rescued by decreasing Myosin II or increasing Patj levels in the ectoderm. We show through segmentation analysis of movies that ectodermal cells near the entering macrophage round up and thus decrease their width; in *eiger* mutants they do so more slowly. These experiments reveal that Eiger and Grindelwald reduce tissue tension through the Crumbs complex to facilitate the ectodermal cell shape changes enabling macrophage invasive migration. Our work supports evolutionary conservation between *Drosophila* macrophage migration into the germband during development and vertebrate immune cell extravasation out of the vasculature during inflammation. We also demonstrate for the first time the *in vivo* effect of a TNFα family member on tissue tension, acting through a novel pathway.

492C β-catenin is a pH Sensor with Decreased Stability at Higher pH *Bree Grillo-Hill*[1], Katharine White[2], Mario Esquivel[2], Diane Barber[2]. 1) Biological Sciences, San José State University, San Jose, CA; 2) Cell and Tissue Biology, UC San Francisco, San Francisco CA.

β-catenin (β-cat) functions as both an adherens junction protein for cell-cell adhesion and as a signaling molecule in the Wnt pathway. A current view is that the key regulator of β-cat stability is the phosphorylation of N-terminal serine and threonine residues to facilitate ubiquitination and subsequent proteasome-mediated degradation. Here, we report that β-cat stability can be regulated by physiological changes in intracellular pH (pHi). We previously showed that overexpression of a Na-H exchanger in the *Drosophila* eye increases pHi by 0.3 units and causes an externally rough eye with underlying defects in cell shapes, contacts and fate misspecifications. This rough eye phenotype is suppressed by overexpression of *armadillo* (*Drosophila* β-cat) and enhanced by *armadillo* shRNA. We found that increased pHi in *Drosophila* retinal tissues and in clonal mammalian epithelial cells significantly decreases β-cat abundance, but has no effect on β-cat phosphorylation. As a mechanism for pH-dependent stability, we found that β-cat association with βTrCP, the E3 ubiquitin ligase that mediates β-cat degradation, is significantly greater when pHi is increased above 7.4. We predict an evolutionarily conserved Histidine (His36) located between the phosphorylated serine residues that are recognized by βTrCP might function in pH sensing, with a neutral His at higher pH enhancing βTrCP binding. This is supported by the published crystal structure of βTrCP complexed with an N-terminal human β-cat peptide which shows close proximity of His36 and Lys365 in βTrCP, with predicted electrostatic repulsion if His36 is charged. To test this prediction, we generated a constitutively charged β-cat$^{H>R}$ mutation and found pHi-independent association with βTrCP and decreased binding, resulting in increased β-cat$^{H>R}$ abundance. This β-cat$^{H>R}$ mutation was identified in multiple samples in the Catalogue Of Somatic Mutations In Cancer database, suggesting a possible role in tumorigenesis. We found that misexpressing β-cat$^{H>R}$ in the *Drosophila* eye induces dysplasia not seen with neutral substitutions, and further induces pronounced tumors which are more severe than

phenotypes observed with characterized oncogenic β-cat alleles such as S33/37A, T41A and S10. Together, our data identify pHi dynamics as a previously unrecognized regulator of β-cat stability, with a mechanism whereby a neutral His36 increases β-cat degradation. Tumorigenic mutations may bypass this pH-sensitive regulation and stabilize β-cat to facilitate cell-cell adhesion and Wnt signaling at the increased pHi of cancer cells.

493A From Gut to Brain: The behavioral consequences of atrazine exposure on the Drosophila microbiome *Siti Nur Sarah Morris*, Kenneth Wan, Sasha Langley, James Brown, Susan Celniker. Environ Genomics & Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, CA.

The triazine herbicide, Atrazine, is widely used by the US agricultural industry; its ubiquitous usage has led to its bioaccumulation in the drinking water reserves of 33 million Americans. Atrazine's pervasiveness is a cause of concern since the potential adverse health effects of the herbicide are not fully understood. Human population exposures are frequently in the low-dose range over a prolonged period of time, making it difficult to draw conclusions about long-term adverse health effects. Furthermore, atrazine has strong effects on microbial communities—extinguishing entire clades whilst promoting growth of others—thus suggesting an unappreciated microbiome component of atrazine susceptibility.

Interestingly, the bidirectional signaling of the gut-brain axis (GBA) indicates a connection between the gut microbiome, brain health and behavior. This suggests that perturbations in the microbiome may adversely affect the host's brain. However, the nuances of the how these prokaryotes influence the brain remain to be elucidated. To that end, *Drosophila* can be used as a model system to interrogate the connection between gut microbiome and behavioral and health outcomes.

We conducted a dose response study at environmentally relevant concentrations of atrazine in *D. melanogater*. This cost-effective screen enables the identification of genes and commensal microbial clades associated with atrazine susceptibility at doses ranging from sub-lethal to chronic toxicity. Flies are assayed for metabolic and behavioral abnormalities and analyzed for the composition of their gut microbiome. These studies pinpoint genes and microbial populations related to atrazine-dependent behavioral abnormalities and health defects. As some behavioral or locomotor phenotypes may be subtle, we employed the cTrax automated behavioral detection system that tracks the movement of fruit flies restricted to two-dimensional surfaces for 5 min intervals. Analysis of this data measures the locomotor fitness, courting frequency, receptiveness of mating and aggression of treated vs. untreated populations. This high-content phenotyping provides substantial statistical power to discern subtle neurological or neuromuscular symptoms.

494B Loss of mitochondrial tRNA processing as a model for mitochondrial disease *Aditya Sen*[1], Agnes Karasik[1], Aranganathan Shanmuganathan[1], Elena Mirkovic[2], Markos Koutmos[1], Rachel Cox[1]. 1) Biochemistry and Molecular Biology, Uniformed Services Univ., Bethesda, MD; 2) Walt Whitman High School, Bethesda, MD, 20817, USA.

Mitochondria are the major ATP producing organelle in the cells. Due to its bacterial origin, human mitochondria contain their own DNA, which encodes for 13 polypeptides, 22 tRNAs and 2 rRNAs. As mitochondrial tRNAs (mt:tRNAs) are embedded within the long polycistronic transcript, proper endonucleolytic processing is necessary to matures the tRNAs. In human, this function is mediated by the mitochondrial RNase P complex which is comprised of three proteins: proteinaceous RNase P (PRORP) or MRPP3, TRMT10C (MRPP1) and SDR5C1 (MRPP2). We used Drosophila as a model system to investigate the underlying mechanism and physiological relevance of this complex. We have identified the orthologous members of mt:RNase P, Mulder (PRORP, CG15896), Scully (MRPP2) and Roswell (MRPP, CG5190), and find that each localizes to mitochondria and can associate with each other. Genetically reducing each member causes mitochondrial dysfunction as supported by highly reduced ATP, the presence of damaged mitochondria, and pupal lethality. Moreover, overexpression of Mulder or Roswell also causes lethality and a decrease in ATP levels. Additionally, we observe that mt:tRNAs are not processed correctly in these mutants, as evident from unprocessed tRNA accumulation. These observations strongly support that the mitochondrial defects we observe are due to a direct consequence of loss of mt:RNase P function. This is the first in vivo evidence showing that defective mt:RNase P causes aberrant mitochondrial tRNA processing, mitochondrial dysfunction and lethality. Fully understanding the role of mt:tRNA processing in vivo is vital because mt:tRNAs encode for only ~9% of the mitochondrial genome, but point mutations in mt:tRNAs are primarily responsible for mitochondrial diseases. This in vivo model will advance our understanding of how defective mitochondrial tRNA processing, arise either from mutations in processing enzymes or tRNAs, impacts human mitochondrial disease.

495C New *Drosophila* reference genomes reveal previously hidden genetic variation *Mahul Chakraborty*, Anthony Long, James Emerson. Department of Ecology and Evolutionary Biology, University of California, Irvine, CA.

Accurate characterization of genetic variation is essential for understanding phenotypic evolution. Typically, high

throughput short reads are aligned to a reference genome to identify the single nucleotide polymorphisms (SNP) and small indels. However, large scale structural mutations (e.g. duplications, deletions, insertions, etc.) are often overlooked by short read based methods. Thus, although such structural variants (SVs) play pivotal roles in genome evolution and the genetic basis of disease, our perception of structural genetic variation is drastically limited by current methods. To overcome these limitations, we resequenced the founder strains of the Drosophila Synthetic Population Resources (www.flyrils.org) using Pacific Biosciences long reads. To shed light on the missing SVs, we constructed *de novo* assemblies for each of these strains. Notably, completeness and contiguity of the assemblies are comparable to or better than the current release of the reference strain, with the majority of the genome represented by contiguous sequences (contigs) measuring 20Mb or longer. Comparisons of these assemblies revealed ubiquitous duplicates, transposon insertions, and inversions, revealing the dynamic nature of genome structure. A large number of these SVs, which were previously unknown, contribute to gene structure polymorphism, expression level variation, and phenotypic adaptations, several of which we describe in detail.

496A Functional Study of Nuclear-Encoded Mitochondrial Duplicated Genes with Testis-biased Expression in *Drosophila melanogaster* *M. Eslamieh*, E. Betran. Biology, University of Texas at Arlington, Arlington, TX.

The analysis of nuclear-encoded mitochondrial genes (N-mt genes) in *Drosophila melanogaster* has shown that most of the duplicated N-mt genes in this species acquired testis-biased expression. These genes tend to be old, often relocated, and also have energy-related functions. These duplicates have been proposed to evolve in response to intralocus sexual antagonism generated by the appearance of a new male-beneficial and female-harming allele initially at a single-copy locus. Since males do not pass the mitochondria to the offspring and they are under intense male-male competition pressures to fertilize females' eggs, selection might favor mitochondria with high-energy production despite the fact that it also will increase reactive oxygen species (ROS) production. Mitochondrial complex I seems to be a good candidate to test this hypothesis because it is not only the biggest respiratory complex but also is one of the primary sources of ROS production. I will present knock down results of N-mt duplicates in complex I (four genes) and discuss the observations. Knockdowns of duplicate and parental are being confirmed by qRT-PCR and phenotypic effects scored. We are planning to knock out these genes to confirm the results and to ectopically express those genes to confirm the level of ROS production.

497B Selection Across Environmental Gradients Leads to the Establishment of Chromosomal Inversions in Populations *Zach Fuller*, Stephen Schaeffer. Biology, Penn State, University Park, PA.

Chromosomal inversions act as negative modifiers of recombination that reduce genetic exchange between heterokaryotypes. It is hypothesized inversions may be favored through the indirect effects of suppressed recombination if they capture sets of locally adapted alleles, thereby preventing exchange with potentially maladaptive alleles. Under models of local adaptation, it is predicted that frequencies will reach fixation in environments where they are favored and replace ancestral recombining haplotypes. However, in nature the frequencies of inversions are often clinally distributed along environmental gradients and heterokaryotypes form at frequencies expected under Hardy-Weinberg equilibrium. Traditionally, the stable equilibria of heterokaryotypes has been explained through coadaptation, overdominance, or the accumulation of recessive deleterious alleles in homokaryotypes. Here, we extend previous models of selection for local adaptation acting on inversions by simulating chromosomes across optimal fitness gradients. By simulating a gradient where extreme phenotypes are favored in local environments, but intermediate traits are favored in transitional habitats, our model simultaneously supports the establishment of homokaryotypes through local adaptation and the stable formation of heterokaryotypes. We find that directional selection acts at either end of the fitness gradient, while overdominant selection acts near the center where intermediate phenotypes are favored. Under all modes of selection and across our simulated environment, the suppression of recombination is favored. Our results support recent empirical observations of extensive *cis*-regulatory differences, additive effects and intermediate levels of gene expression in third chromosome heterokaryotypes of *Drosophila pseudoobscura*. These models may explain a process through which chromosomal inversions can initially establish across a heterogeneous environment.

498C Transposable element expression over embryonic development in male and female embryos of *Drosophila pseudoobscura* and *D. miranda* *Lauren Gibilisco*, Doris Bachtrog. Department of Integrative Biology, University of California, Berkeley, CA.

Transposable elements ("TEs") are mobile genetic elements whose movement can be deleterious to their hosts. Host genomes have evolved multiple strategies to suppress TE mobilization, including targeting by small RNAs and heterochromatin formation. In the germline and during early embryogenesis, TE repression is particularly important because any activity can cause new, heritable insertions. We characterized TE expression during the maternal-to-zygotic transition in male and female embryos of *Drosophila pseudoobscura* and *D. miranda* and found higher repeat expression during early development in males compared to

females. We hypothesize that the Y chromosome influences heterochromatin establishment and sex-specific TE expression profiles during early development.

499A The recombination landscape of *Drosphila virilis* under hybrid dysgenesis *Lucas Hemmer*, Justin Blumenstiel. Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS.

Recombination is a form of DNA repair using homologous sequences or chromosomes. It frequently takes place in meiosis through programmed double-stranded breaks resulting in a crossover or a gene conversion. Although mitotic homologous repair is less frequent in mitotically dividing cells, recombination can be used to repair DNA damage from exogenous sources, such as transposable elements. It is still unclear what the relationship is between the frequency of double-stranded breaks, both endogenous and exogenous, and the decision to repair the DNA through one of many pathways, including crossing over and gene conversion. It appears that the decision may be partially based on where the DNA damage occurs along the length of a chromosome and as well as a genetically determined minimum and maximum level of tolerable crossing over to maintain genomic integrity. We aim to use the *Drosophila virilis* hybrid dysgenesis model to understand how recombination landscapes change under transposable element proliferation. In this system, a mating event between two strains of *D. virilis* with divergent transposable element loads will result in the hybrid dysgenesis phenotype, which includes increased DNA damage in the germline, reduced fertility or sterility, and aberrant male recombination. Only one direction of the cross will result in hybrid dysgenesis and allow us to examine recombination in genetic identical F1 females; half with baseline levels of programmed DNA damage and the other half with an increased and persistent level of DNA damage resulting from transposable element proliferation. We are using multiplexed shotgun genotyping to map crossover events along the genome and comparing the recombination landscape between hybrid dysgenic and non-hybrid dysgenic individuals. Finally, we are examining whether the crossovers are mitotic or meiotic in origin by looking for consistent genotypic patters in the progeny of dysgenic individuals.

500B The impact of regional climate variables on genome size evolution in *Drosophila* species *Carl Hjelmen*, J. Spencer Johnston. Department of Entomology, Texas A&M University, College Station, TX.

Genome size has been found to vary extensively among organism, with up 7,000 fold variation in animals. This extreme amount of variation has not been found to be related to correlate with complexity, but has been found to be largely due to increased regions of noncoding DNA. There have been many hypotheses for this variation in noncoding DNA, ranging from low effective population sizes to climatic adaptation. Based on Bergmann's Rule of increasing body size with increasing distance from the equator, it is hypothesized that genome size should increase with decreasing temperature. Increased genome size has been shown to lead to increased cell size, which then can result in increased size of an organism. In order to investigate the impact of climatic variables on genome size evolution, I have compared genome size with climatic data for over fifty species of *Drosophila*. Genome sizes were estimated using dissected neural tissues stained with propidium iodide that was subsequently analyzed with flow cytometry. Climate information, including average temperate, high/low temperature, precipitation, latitude, etc., was collected from literature. These relationships were tested with traditional methods of analysis, such as regression analysis, and also tested with phylogenetic contrasts. While some relationships of genome size with some of the climate variables were found, when analyzed with phylogenetic methods, this pattern disappeared, suggesting phylogenetic relationships impact both genome size variation and species climate tolerance.

501C Functional analyses of the transposable element-derived genes *DPLG1* and *DPLG4* in *Drosophila melanogaster* *D. Jangam*[1], C. Feschotte[2], E. Betrán[1]. 1) University of Texas at Arlington, Arlington, TX; 2) University of Utah School of Medicine, Salt Lake City, UT.

Several domesticated transposases from *PIF/Harbinger* DNA transposable elements (TEs) have been described in *Drosophila* by Betrán and Feschotte labs. Here, we present work aimed to elucidate the function of two *PIF/Harbinger* derived transposases named *Drosophila PIF like gene 1* (*DPLG1*) and *DPLG4*. GO enrichment analysis of *DPLG1* and *DPLG4* co-expression cluster, as defined by a recent high-resolution analysis of the developmental transcriptome of *D. melanogaster* by Graveley *et al.* 2011, shows these genes co-express with transcription factors and the study by Casola *et al.* shows that these genes have retained their HTH DNA binding domain but have lost their DDE catalytic. Thus we postulate that these proteins are domesticated for their ability to recognize and bind specific DNA sequences and are now transcription factors. Additionally, since TE proteins are able to recognize TE sequences, we further hypothesize that these genes may be potentially domesticated to defend against transposition events.

Taking advantage of the UAS/GAL4 system, *DPLG1* was knocked down in all tissues using *Actin5c-GAL4* driver and a UASt-RNAi-*DPLG1* line, and we performed RNA-Seq analysis of the *DPLG1* knockdown (KD) testis. The testis of these KD males show increased activity of LINE TEs including all three telomeric elements. QRT-PCR analyses of these samples showed significant difference only in the most differentially expressed TEs. We have recently generated null mutants of *DPLG1,* in collaboration with Buszczak lab, and we will be testing if the effect in

the *DPLG1* Knockout (KO) flies would be amplified from what we observe in the KD testis. *DPLG4* might also be also involved in TE control. A study by Handler *et al.* showed that *DPLG4* scored positive in a large RNAi screen for new ovarian piRNA factors. In a screen of over 7000 genes, *DPLG4* was one of the 368 genes that scored positive for upregulation of gypsy-lacZ reporter in the ovarian somatic cells. We have evidence which shows that DPLG4 localizes in the nucleus of the female germline and thus, could potentially be interacting with DNA to regulate TE activities. We have also generated a null mutant of *DPLG4,* also in collaboration with Buszczak lab, which we will be using to further investigate the role of *DPLG4* in TE regulation. Additionally, our data from the KO and KD experiments shows that *DPLG4* is important for the viability, and possibly fertility in *D. melanogaster.*

502A Lessons learned from an examination of genome size variation in inbred lines of D. melanogaster *J. Spencer Johnston*[1], Aaron Tarone[1], Carl Hjelmen[1], Erin Kelleher[3], Stuart Macdonald[2]. 1) Dept of Entomology, Texas A&M University, College Station, TX; 2) Dept. Molecular Biosciences, Univ. Kansas, Laurence, KS; 3) Dept. Biology and Biochemistry, University of Houston, Houston, TX.

An examination of genome size in the 204 inbred lines of the DGRP (Drosophila Genome Research Project) revealed significant among line genome size variation. Lines selected from the extreme ends of this distribution showed a relationship between genome size and pupal survival, adult survival, pupation rates, female pupal mass, and female eclosion rates. The DGRP lines were derived from a single population in Raleigh, N. C. In contrast, the DSPR recombinant inbred lines (RIL) were developed following 50 generations of random mating among 8 source populations. We report here genome size for 204 of the DSPR lines. The variation among the DSPR lines was twice that that observed for the DGRP lines. In part, the increased variation reflected genome size variation among the founding populations. However, a number of the DSPR lines had genomes that exceeded that of any of the founders by as much as 35 Mbp. These extreme genotypes could not easily be explained by transgressive segregation of elements existing in the source populations. There were no comparable extremes at the lower end of the distribution. Rather, the large genomes appear to reflect genome size expansion associated with mobile elements. Crosses involving lines with larger genomes frequently showed reduced ovarian development typical of hybrid dysgenesis. A GWAS (Genome Wide Association Study) of genome size from the DGRP had identified a QTL that included the PIWI element Flamenco. With this in mind, we examined stocks containing a mutant for a number of PIWI genes, including three Flamenco mutant stocks. The average genome size of each the Flamenco mutant stocks was exceptional. All were large with multiple exception individuals that had significantly expanded genomes. We are currently increasing the number of DSPR lines scored for genome size in an effort to provide power for a GWAS to seek an association of Flamenco and genome size in the DSPR strains.

503B Investigating the function of *Drosophila* zinc finger genes in transposable element silencing *Bhavatharini Kasinathan*[1,2], Kami Ahmad[2], Harmit Malik[2,3]. 1) Medical Scientist Training Program, University of Washington, Seattle, WA; 2) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Howard Hughes Medical Institute.

Eukaryotic genomes are plagued by transposable elements and their mobilization poses a threat to fertility and genome integrity. As a result, eukaryotes have developed an arsenal of mechanisms to keep transposable elements in check within the genome. Vertebrates contain a stunning example of such a mechanism, KRAB zinc finger proteins. These proteins have been finely tuned to recognize, bind and repress transposable elements in a species-specific manner through a series of lineage specific expansions and diversifications. However, there has been no system analogous to KRAB zinc fingers found in invertebrates. We report an analysis of a cluster of genes encoding C2H2 containing zinc finger proteins with zinc finger associated domains (ZAD) that has undergone lineage specific expansion in *Drosophila.* Strikingly, we find that the protein produced by the evolutionarily young member of the gene family binds to specific foci in the chromocenter while the parental protein localizes diffusely throughout the nucleus. The expression pattern shows that the evolutionary young gene and the parental gene are subject to distinct transcriptional regulation. Additionally, analysis in *D. melanogaster* reveals that while the parental gene overexpression has no effect on fertility and viability, misexpression of the evolutionarily young gene is lethal during development. To assess the essentiality of these two genes, we are creating knock out alleles using CRISPR technology. We predict that this cluster of ZAD-containing C2H2 zinc finger proteins play a key role in suppressing transposable element mobilization in *Drosophila*

504C The geographic and haplotypic structure of polymorphisms in centromeric regions. *Charles H. Langley*[1], Sasha A. Langley[2], Gary K. Karpen[2,3]. 1) Department of Evolution and Ecology, University of California - Davis, Davis, CA; 2) Lawrence Berkeley National Laboratory, Life Sciences Division; 3) Lawrence Berkeley National Laboratory, Life Sciences Division and University of California - Berkeley, Department of Molecular and Cell Biology.

The repetitive nature of DNA in the heterochromatic centromeric regions of *D. melanogaster* compounded with low gene density and low rate of recombination continue to be significant barriers to genetic and evolutionary analysis. Nevertheless the improvements in the recently released genome assembly (R6), continuing discussion of

diverse evolutionary theories and the many advances in the functional biology of heterochromatin and centromeres make the characterization of genomic diversity in these heterochromatic regions an ever more compelling goal. What are the patterns linkage disequilibrium and the structures of common haplotypes? Is there evidence of recombination? Particularly in the 'centromeric gaps?' Are haplotypes encompassing the centromeric gap associated with variation in satellite DNA sequences? And do these pattern elucidate the evolutionary dynamics of centromeric regions? To address these and other questions we applied simple and stringent filters to GATK 'assemblies' of two samples of genomes, DPGP2 (113, across Africa) and DPGP3 (187, a single population in Zambia). The filtering included Q50, no indels, scored in every genome, minimum allele frequency of 0.10 and the removal of many SNPs that exhibit a 3-locus linkage disequilibrium pattern with an expected signature of random genotyping error. The patterns of pairwise linkage disequilibrium across the heterochromatic regions of chromosomes 2 and 3 exhibit a large dynamic range and reveal blocks of more extreme values. Clustering the haplotypes comprised of the thousands of SNPs surviving the filtering identifies many common large-scale haplotypes. While haplotypes apparently derived by exchange between common haplotypes can be identified, there is little evidence for exchange in or near the centromeric gaps. Thus each sampled chromosome falls into a centromeric haplotype, or *cenhap*, that includes the unknown sequences in the gap. The 'out of Africa' *cenhaps* of each chromosome cluster with a single small African clade consistent with a severe bottleneck effect. The *cenhap* diversity can be leveraged to map other sequence variation. SNPs in unmapped contigs of the R6 assembly can be associated with particular chromosomes through linkage disequilibrium with *cenhap* SNPs. Variation in the counts of kmers of known and novel satellite DNA sequences can be associated with *cenhaps*. For example, the counts of *Responder* locus derived kmers varies strongly among chromosome 2 *cenhaps*.

505A Functional Analysis of *Dntf-2r* and *Ran-like* Nuclear Transport Retrogenes in *Drosophila melanogaster.* *A. Mirsalehi*, D. Jangam, S. Domingues, E. Betran. Biology, University of Texas at Arlington, Arlington, TX.

Dntf-2 and *Ran* are two nuclear transport genes. Retroduplicates of *Dntf*-2 and *Ran,* called *Dntf-2r* and *Ran-like* exist in *D. melanogaster* are expressed nearly exclusively in the male germline. To elucidate the reasons for the selective pressures that lead to recurrent duplication of *Dntf-2* and *Ran* (i.e., the same retroduplications have been observed in other lineages) we are studying the function of *Dntf-2r* and *Ran-like* in *D. melanogaster*.

Whole mount *in situ* hybridizations were performed using specific probes for parental and retrogenes to compare the details of testis-specific expression of *Dntf-2* and *Ran* with their correspondent retrogenes. The protein localization of *Ran-like* and *Dntf-2r in D. melanogaster* has been observed in transformed lines with constructs of these genes fused to Red Fluorescent Protein (RFP) for *Ran-like* and Green Fluorescent Protein (EGFP) for *Dntf-2r*. Those results point to an array of complex functions for these genes during spermatogenesis.

A detailed study of the effects of knocking down *Dntf-2r* and *Ran-like* retrogenes in male germ-line has been performed. Double-stranded RNA-mediated interference (RNAi) technique was implemented and lines from VDRC for *Dntf-2r* and *Ran-like* were used to knockdown these genes. Using phase contrast and fluorescent microscopy, defects in the testis of knockdown flies have been studied. Knockdown crosses were performed using RNAi driven by the GAL4/UAS system in particular cells. Fertility assays were performed with the progeny from RNAi crosses. Taking advantage of CRISPR/Cas9 technique combined with homologous recombination knockout mutants have been generated for *Dntf-2r* and *Ran-like* where the gene has been replaced by a RFP driven in the eye. Results from the knockdown and knockout of those genes will be discussed.

506B A major role for retrotransposons in the expansion of the *Drosophila ananassae* F element *Laura K. Reed*[1], Wilson Leung[2], Christopher D. Shaffer[2], Sarah C.R. Elgin[2]. 1) Dept. of Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Dept. of Biology, Washington University in St. Louis, St. Louis, MO.

The discordance between genome size and the complexity of eukaryotes can partly be attributed to differences in repeat density. The heterochromatic Muller F element (~5.2 Mb) is the smallest chromosome in *Drosophila melanogaster* but it is substantially larger (>18.7 Mb) in *Drosophila ananassae*. To assess the impact of this expansion on gene characteristics, we improved the sequence quality and annotated the genes in a 1.4 Mb region of the *D. ananassae* F element, as well as a 1.7 Mb region from the euchromatic D element for comparison. We find that transposons (particularly LTR and LINE retrotransposons) are the major contributors to this expansion, accounting for 78.6% of the *D. ananassae* F element. By contrast, horizontal gene transfer of *Wolbachia* sequences to the *D. ananassae* genome plays only a minor role (~0.02%). The properties of F element genes are distinct from D element genes (*e.g.*, larger coding spans, larger introns, more coding exons, lower codon bias) in both *D. melanogaster* and *D. ananassae*, but these differences are exaggerated in *D. ananassae*. Despite differences in gene characteristics and the surrounding repeat density, RNA-Seq analyses show that *D. ananassae* F element genes have a similar range of expression levels compared to genes in euchromatic domains. *D. ananassae* F element genes show greater deviations from uniform codon usage and less optimal codon usage compared to *D.*

melanogaster F element genes, which suggests that more of the codon bias in *D. ananassae* F element genes can be attributed to mutational biases instead of selection. This study provides insights into how transposons can affect genome size and how genes can function within a highly repetitive domain. Supported by NSF grant #1431407 and HHMI grant #52007051.

507C Temporally Varying Selection and its Influence on Genetic Variation *Alyssa Bangerter*, Alan Bergland. Biology, University of Virginia, Charlottesville, VA.

Organisms experience environments that change over space and time and adapt to this heterogeneity. *Drosophila melanogaster* rapidly adapts to seasonal change and exhibits significant shifts in allele frequency from spring to fall. However, we do not know the fine-scale patterns of these allele frequency shifts and how it affects patterns of linkage disequilibrium. Are allele shifts rapid at the turn of the seasons, or is it a continual, gradual shift? Do the patterns of linkage disequilibrium change in response to the strength and timing of seasonal selection? Using previously identified seasonally oscillating SNPs, I can track the fine-scale oscillations in SNP frequency by sequencing bi-weekly wild *D. melanogaster* collections from a focal orchard. After identifying the patterns of allele frequency shifts, I will also see if the shifts correlate with potential environmental selective pressures such as temperature and humidity. The sequenced wild *D. melanogaster* genomes will also be phased to track patterns of linkage. Using D' polarized by the seasonality of SNPs, I will show if patterns of linkage disequilibrium change throughout the season to maintain sets of functional seasonally oscillating SNPs and if LD increases rapidly in response to rapid seasonal changes in selection pressures.

508A The role of detoxification gene *Cyp12d1* in resistance to caffeine in *Drosophila* *Dianarys Hernandez-Aquino*, Stuart J. Macdonald. Molecular Biosciences, University of Kansas, Lawrence, KS.

Xenobiotic compounds, for instance those produced by plants as a defense against herbivory, as well as a wide variety of agricultural pesticides, are a constant challenge for animals. To help defend against these toxins animal genomes harbor numerous detoxification genes, and these loci can segregate for functional variants that affect the efficacy of detoxification. Previously, we used the *Drosophila* Synthetic Population Resource (DSPR), a series of recombinant inbred lines derived from a multiparent intercross, to map loci contributing to resistance to caffeine, a model xenobiotic compound. Through high-resolution mapping, RNAseq, and RNAi expression knockdowns we found evidence that the cytochrome P450 gene *Cyp12d1* is associated with resistance to caffeine. To further our understanding of gene function we have performed additional RNAi experiments, knocking down the expression of *Cyp12d1* in various tissues relevant to detoxification. We found that the adult midgut is a major site of caffeine detoxification through *Cyp12d1. Cyp12d1* is subject to copy number variation in both laboratory and natural populations of *Drosophila melanogaster*. Several studies have shown that individuals with higher copy numbers of particular detoxification genes have an increased resistance to certain xenobiotic compounds, and we found tantalizing evidence that *Cyp12d1* copy number is associated with caffeine resistance in the DSPR. To build upon, and validate this work we are carrying out targeted association studies to precisely test the effect of *Cyp12d1* copy number on caffeine resistance. The large, outbred populations we employ provide additional resolution over the DSPR, and additionally survey a broader range of natural variation. Finally, to directly assess the effect of *Cyp12d1* on caffeine resistance, we have used CRISPR-Cas9 gene editing to generate two independent putative loss-of-function mutations in *Cyp12d1* (a 2-bp, and a 16-bp deletion), and are in the process of testing these mutations for their effect on the trait. Our ultimate goal is to extend our genome editing to manipulate *Cyp12d1* copy number while controlling the genetic background, directly testing the hypothesis that copy number of *Cyp12d1* causally influences resistance to caffeine. The role of copy number variants in both evolutionary adaptation and disease are major topics of study, however many gaps remain in our understanding of how such variants affect phenotypes. Our research will provide further insight into the functions of copy number variants, and the genetic basis and mechanisms of xenobiotic resistance in general.

509B Effects of saturation deficit on water balance related traits in seasonal populations of a tropical drosophilid-*Zaprionus indianus* *Bhawna Kalra*, Ravi Parkash. Department of Genetics, Maharashi Dayanand University, Rohtak, India.

Seasonally varying populations of ectothermic insect taxa from a given locality are expected to cope with simultaneous changes in thermal and humidity conditions through phenotypic plasticity. Accordingly, we investigated effect of saturation deficit on desiccation resistance in wild caught flies from four seasons (spring, summer, rainy and autumn) and corresponding laboratory flies reared under season specific simulated thermal and humidity growth conditions. Summer flies showed about three times higher desiccation resistance and cuticular lipids compared with rainy season flies grown under simulated season specific thermal and humidity conditions. In contrast intermediate trends were observed for water balance related traits in flies reared under spring or autumn specific conditions but trait values overlapped across these two seasons. Further, a three fold difference in saturation deficit (an index of evaporative water loss due to combined thermal and humidity effect) between summer (27.5 mB) and rainy (8.5mB)

season associated with two fold differences in the rate of water loss. Higher dehydration stress due to highest saturation deficit in summers is compensated by storage of higher amount of energy metabolite (trehalose) and cuticular lipids and these traits correlated positively with desiccation resistance. In *Z. indianus*, observed changes in desiccation related traits due to plastic effects of simulated growth conditions correspond to similar changes exhibited by seasonal wild-caught flies. Our result show that developmental plastic effects under ecologically relevant thermal and humidity conditions can explain seasonal adaptations for water balance related traits in *Z. indianus* and are likely to be associated with its invasive potential.

510C Probabilistic invasion dynamics in the fly gut microbiota *Benjamin Obadia*, Tüzün Güvener, Vivian Zhang, William Ludington. Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Individuals are constantly faced with new potential invaders of their resident gut microbiota, but only some strains successfully establish themselves. We sought to understand to what extent this variability is probabilistic (i.e., due to chance) *versus* determined by specific host and bacterial traits. Our investigation was done in the context of a defined bacterial community of Acetobacteraceae and Lactobacillaceae in germ-free, gnotobiotic, and conventionally-reared *Drosophila*. Using a modified CAFE assay, we delivered precise doses of bacteria to hundreds of individual flies and measured their probability of successful colonization and their growth rate in the fruit fly gut. We focused on three isolates of *Lactobacillus plantarum* (wild fly, lab fly, and human isolates), a known probiotic having positive impacts on the intestinal physiology and immunity. We quantified fitness and niche differences *in vitro* and in the fly gut, finding minor differences in both. Microscopy analyses indicate that *Lactobacillus* is capable of colonizing distinct regions of the fly gut. Notably, the wild fly isolate had a greater colonization probability, a greater population size, and lower variation in population size than the other ones.

We conducted experiments to determine the sources of the variation between individual flies and between the different bacterial strains. In invasions of conventionally-reared flies, we find that variance in the resident microbiota population increases with increasing input dose of commensal bacteria, indicating that invasion destabilizes the gut microbiota and suggesting that host-microbiota feedbacks may affect population dynamics.

Overall our data reveal that stable intestinal colonization by bacteria is a probabilistic event following a lottery model with a bottleneck effect where invader-host feedbacks induce transitions between two stable states (colonized versus uncolonized). The results have implications for not only gut microbiome stability but also for macro-ecological invasions where large variation exists in the success of an invasion.

511A Genetic structure of *Drosophila mediopunctata* populations in remnants of Brazilian Atlantic forest using microsatellite markers located on chromosomes without inversions *Rafael ES Penha*, Louis B Klaczko. Departamento de Genética, Evolução e Bioagentes , Unicamp, Campinas, São Paulo, Brazil.

Drosophila mediopunctata is an important model of population and ecological genetics. The present study was aimed at assessing the genetic variation and population structure of *D. mediopunctata* populations in six Atlantic forest remnants, using microsatellite markers (SSR) located on chromosomes III and V, because they do not have chromosomal inversions (usually under selection). Thus, we analyzed samples from six populations distributed in forest patches between the states of São Paulo and Rio de Janeiro, Brazil; they were: Teresópolis (TE); Itatiaia (IT); Colinas do Atibaia (CA); Costa e Silva (CS); Santa Genebra (SG); and Capivari (CV). We used 12 microsatellite loci in the analysis: five located on chromosome III and seven on chromosome V. The genotyping was performed by analysis of fragments in an automatic sequencer. The analysis of the effective population size showed that all populations are infinitely large (except TE: Ne = 238 by Linkage Disequilibrium method). In relation to the genetic diversity of studied populations we found 1018 distinct alleles, the averages of the values of allelic richness varied from 13.3 (TE) to 15.2 (SG). The mean expected heterozygosity in all populations varied little, from 0.84 (IT) to 0.87 (SG). The total mean expected heterozygosity was 0.85, however in all populations we observed a deviation from Hardy-Weinberg Equilibrium and a high value of F_{IS}, which ranged from 0.10 (IT) to 0.23 (CS), which shows that there is an excess of homozygotes in the samples. Null alleles may explain these results. Only a single locus didn't show null alleles; when we estimated the frequency of null alleles per population, it ranged from 0.04 (IT) to 0.12 (CS). However, other factors can explain these observations: the influence of natural selection, or inbreeding. The global genetic structure obtained for the F_{ST} was 0.017 ($P < 0.0001$), which means that there is a low but significant degree of genetic structuring. For the pairwise comparisons, the highest value was between CS and CV (0.03; $P < 0.0001$) and the lowest was between CS and SG (0.0009; $P > 0.05$). The number of migrants among populations ranged from 10 (CS and CV) to 27 (CS and SG). These results show that the species has a high genetic diversity and a low genetic structure, whereas there is ample gene flow among fragments. Acknowledgement: G. Ananina; MRD. Batista; MT. Brianti; R. Cavasini; MS. Couto; AB. Martins; FB. Rocha; D. Moraes; GM. Mori; PR. Laborda; IM. Ventura for collecting help; SH. Sofia for providing technical facilities. Funding Agencies: CAPES, CNPq, FAEPEX-UNICAMP, FAPESP.

512B Functional genomic landscape reveals population differentiation and adaptation in reproductive proteins *Craig Stanley*, Alexandra Jones, Rob Kulathinal. Biology, Temple University, Philadelphia, PA.

As a functional class, reproductive genes generally encode some of the most divergent proteins between closely related species. Yet, while signatures of divergence and selection are well characterized at the species level, little is known about the divergence of reproductive genes in initial population level differentiation, and how these changes drive the initial barriers. Here, we annotate ten subclasses of reproductive proteins in males and females in deep-sequenced populations of *Drosophila melanogaster* to understand the role of selection in population divergence. Male-specific reproductive genes show an overall enrichment of positive selection in contrast to genes expressed in either non-reproductive specific genes or female-specific reproductive genes. When comparing two populations of *D. melanogaster*, sperm-specific genes were found to be the only male subclass to exhibit significantly higher population differentiation, Fst. However, of the top five most differentiated genes, we identify only one positively selected gene. These results point to the important interplay of selection and drift in driving early stages of speciation and the preferential role of proteins involved in sperm motility.

513C Introgression-derived segregation distortion promotes unlikely gene flow *Christopher Leonard*[1], Zachary Fuller[2], Randee Young[1], Stephen Richards[3], Stephen Schaeffer[2], Nitin Phadnis[1]. 1) University of Utah, Salt Lake City, UT; 2) Pennsylvania State University, College Station, PA; 3) Baylor College of Medicine, Houston, TX.

Segregation distorters are chromosomes that promote their own enrichment in the meiotic pool of their host, and are classic examples of selfish alleles. Distorters are found nearly ubiquitously in eukaryotes and have been proposed as an explanation for enduring evolutionary phenomena including the rapid evolution of meiotic genes, meiotic sex chromosome inactivation, and the development of hybrid incompatibilities leading to speciation.

The Sex-Ratio chromosome of *Drosophila persimilis* is a paradigmatic example of a distorting X chromosome. Carrier males produce almost exclusively X-bearing sperm, dramatically biasing the sex ratio of their progeny toward females. The Sex-Ratio (SR) chromosome contains a large inversion relative to the *D. persimilis* standard (ST) X chromosome. Strangely, the ST chromosome carries the derived inversion, while the SR chromosome is collinear with the chromosome from its sister species *D. pseudoobscura* (Dpse). This oddity was first noted nearly eighty years ago, but has remained a cause for speculation since. The simplest explanation for the collinearity between SR and Dpse is that the SR chromosome may have been derived through re-inversion at the same breakpoints to restore the ancestral orientation.

By collecting new SR strains, we confirmed the collinearity between SR and Dpse. Collinearity extends to the base pair, indicating that if a reinversion occurred, it was improbably precise. A sliding window phylogeny test across the X chromosome shows that SR clusters with Dpse at the inversion breakpoints, where hindered recombination between SR and ST preserves the evolutionary history of SR. We propose that this cross-species clustering is the result of a Dpse introgression that occurred approximately 300kya and founded the SR chromosome.

The role of distortion in speciation becomes cloudier. Large regions of the *D. persimilis* SR chromosome retain their ancestral *D. pseudoobscura* identity, despite conditions that should prevent such gene flow. These regions exist on a rapidly evolving X chromosome, and are associated with an inversion that contains hybrid incompatibility loci. We propose a diverse role for distortion in the formation of species, both as a pressure for the development of hybrid incompatibilities, but also as perhaps one of the only forces that can rupture the barriers to gene flow that distortion itself may have helped form.

514A Investigating the chromosomal and nuclear organization of the Neo-Y chromosome of *Drosophila Miranda* with Hi-C *Kevin Wei*, Emily Brown, Doris Bachtrog. Intergrative Biology, University of California Berkeley, Berkeley, CA.

The differentiation of sex chromosomes typically entails dosage compensation of of one chromosome and degeneration of the other. Degeneration is typified by pseudogenization, cessation of recombination, and transposable elemenet insertion. Furthermore, the degenerate chromosome acquires a heavily repressed chromatin state that is highly compact and inaccessible, known as heterochromatin. The Y chromosome shared by most Drosophila species is thought to have formed ~50 MYA and is now extremely gene-poor and repeat-rich. Due to the low sequence complexity, our understanding of the heterochromatic state of the Y has been largely restricted by coarse analyses, despite the plethora of genetic and molecular tools to characterize chromatin states at high resolution. *D. miranda* has a newly derived Y chromosome that emerged around 1 MYA, which has thus far maintained recognizable homology with the Neo-X, while accumulating many transposable element insertions and pseudogenes. To understand the epigenetic organization of the neo-Y heterochromatin, we performed high resolution and genome-wide chromosome conformation capture (Hi-C) which identifies regions of the genome that are in close proximity in the nucleus. Due to the highly compact nature of heterochromatin, we expect the neo-Y sequences to be strongly associated with itself. Furthermore, we will determine the relationship between transposable element insertions and the local chromatin organization, we expect that insertions are surrounded by more compact chromatin environment. This approach not only sheds light on the regulation and organization of the Y chromsome, it

also provides a snap shot of the evolution of the chromesome and the molecular processes accompanying degeneration.

515B Molecular changes responsible for *de novo* gene origination *Logan K Blair*, Olga Barimina, Artyom Kopp. Evolution and Ecology, UC Davis, Davis, CA.

Genome comparisons indicate that every species has some unique genes. While most of these evolve from genes that are pre-existing, a few evolve *de novo* from non-genic sequences. *De novo* gene origin constitutes the most radical form of genetic novelty, yet we do not understand what genetic changes convert "inert" genomic regions into functional genes. Here, I test the molecular mechansims responsible for activating existing open reading frames (ORFs) in natural populations of *Drosophila melanogaster*. Specifically, I test (1) whether ORF expression is caused by the evolution of an entirely new cis regulatory element or (2) whether sequence with the latent potential to be a *cis* regulatory sequence already exists, but is hidden inside condensed chromatin; the expression of the latent ORF is then caused by a change in local chromatin structure, which uncovers the *cis* regulatory sequence. To do this, I am obtaining chromatin-state profiles using ATAC-seq and determining native regulatory activity of regions surrounding ORF expression through reporter assays. These techniques will demonstrate whether novel regions of open chromatin and entirely new *cis* regulatory elements, respectively, are derived states present only in genotypes which express novel ORFs.Together, this information will fill in a critical gap in our understanding of how new genes evolve.

516C Resolving Copy Number, Order, And Expression Differences Within Highly-Similar, Tandemly-Repeated Multigene Families: *Sdic*, A Case Study *Bryan Clifton*[1], Pablo Librado[2], Edwin Solares[1], Xiao-Qin Xia[3], Antonio Marco[4], Julio Rozas[5], Jose Ranz[1]. 1) Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA, USA; 2) Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark; 3) Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, China; 4) School of Biological Sciences, University of Essex, Colchester, United Kingdom; 5) Departament de Genetica, Microbiologia i Estadistica, and Institut de Recerca de la Biodiversitat, Universitat de Barcelona, Barcelona, Spain.

Recent expansions of tandem gene duplicates are hotbeds for the evolution of novel gene expression patterns and the exploration of new functional niches. However, copy number and sequence differences among copies within these regions are typically poorly resolved using short sequencing read-based techniques and current genome assembly pipelines due to high sequence identity among paralogs. A model of such region is *D. melanogaster*-specific *Sdic* (*Sperm-specific dynein intermediate chain*), the expression of which has been linked to increased post-mating sexual selection through sperm competence. Using various types of massively parallel sequencing data, we studied the organization, sequence evolution, and functional attributes of the different *Sdic* paralogs. Leveraging long sequencing read data, we uncovered both copy number and order differences relative to the currently accepted *Sdic* region annotation in the *D. melanogaster* reference genome, and gained new insights into the structural variation of this region across strains from different geographical origins. Two signatures of diversifying selection were detected in the reference genome, which, despite evidence of pervasive gene conversion between paralogs, have contributed to the evolution of a variety of C-termini and miRNA binding site compositions. Scrutiny of 59 RNA-Seq datasets from different biological conditions revealed distinctive expression breadths among the paralogs, with three paralogs showing unforeseen expression in females. Our results strongly suggest that the genome consolidation of the *Sdic* region is more the result of a quick exploration of different paths of molecular tinkering by different paralogs than a mere dosage increase, which could be a recurrent evolutionary outcome in the presence of persistent sexual selection. These results also highlight the critical importance of enhanced long sequencing read-based assemblies in correctly resolving the structural features of highly-similar tandemly-repeated regions, which are undercharacterized despite their huge potential for playing a key role in adaptive evolution.

517A The fourth chromosome's impact on genome-wide patterns of gene expression *Christopher M. Kimber*, Martin Johnsson, Dominic Wright, Urban Friberg. IFM Biology, Linköping University, Linköping, Sweden.

The fourth chromosome, being non-recombining, largely heterochromatic and relatively gene-poor, remains relatively poorly understood in comparison to the rest of the genome. These distinctive characteristics do, however, raise the possibility that it may interact with the rest of the genome in a unique way. To examine the fourth chromosome's impact on genome-wide patterns of gene expression, we study the consequences of whole-chromosome manipulations of gene expression on the fourth chromosome itself. We find that the fourth chromosome has an extremely small relative impact on patterns of gene expression genome-wide, when compared to the impacts of typical autosomal regions. We consider the implications of this limited impact in an evolutionary context, with respect to the fourth chromomsome's nature as a non-recombining region and as an ancient sex chromosome.

518B Has *jim* contributed to wing shape divergence in Drosophila? *Jennifer Lachowiec*[1], Ricardo D'Oliveira Albanus[2], Richard Lusk[1], Ian Dworkin[3], Patricia Wittkopp[1], Joseph Coolon[4]. 1) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 2) Program in Biomedical Sciences, University of Michigan, Ann Arbor, MI; 3) Department of Biology, McMaster University, Hamilton, Canada; 4) Department of Biology, Wesleyan University, Middletown, CT.

Divergence in gene expression during development can lead to phenotypic differences between species. The sister species *Drosophila simulans* and *D. sechellia* exhibit several phenotypic differences, including subtle changes in adult wing morphology. To quantify divergence in gene expression during wing development, we performed RNA-seq on wing imaginal discs. We observed high conservation of gene expression but also identified a number of key transcription factors with divergent levels of expression. To categorize the regulatory changes underlying this divergent gene expression, we also performed RNA-seq on wing imaginal discs from F1 hybrids between *D. simulans* and *D. sechellia*. The majority of divergence was due to *cis*-regulatory changes between the species with only ~80 genes with expression divergence due to *trans*-regulatory changes. Sequence analysis of the putative regulatory sequences upstream of these genes revealed a massive enrichment for binding site motifs of the transcription factor *jim. Jim* expression and coding sequence were not conserved between *D. simulans* and *D. sechellia*, suggesting that changes to this lone transcription factor could explain the majority of divergence in gene expression among genes with *trans*-regulatory changes. Furthermore, mutations to *jim* have previously been shown to subtly affect wing shape in *D. melanogaster*. This study identifies *jim* as a strong candidate gene for the divergence of wing shape between *D. sechellia* and *D. simulans*.

519C Precise genetic basis of quantitative function evolution of the model gene *Alcohol dehydrogenase* *David Loehlin*, Sean Carroll. Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

All genes are potentially under selection for optimal function. Yet despite this ubiquity, genetic priniciples behind quantitative changes in gene function are not known. What aspects of gene structure are alterable by mutation and selection to change function, and is there a predominant mode of function evolution? To begin to find answers to these questions, we dissected the nucleotide changes underlying a classic model of quantitative evolution: the "fast" and "slow" alleles of the *Alcohol dehydrogenase* (*Adh*) gene in *Drosophila melanogaster*. We build on pioneering work by Cathy Laurie and colleagues, using comparative transgenic methods to functionally identify the suite of causative nucleotide substitutions underlying this adaptive polymorphism. While changes to the protein structure do make a contribution, we find that the predominant mechanism (both in magnitude of effect and number of substitutions) is cis-regulatory substitutions located in promoter sequence. Further, we find repeated substitutions at some of these same sites in other *Drosophila* species with diverged ADH enzyme activity, suggesting the possibility of hotspots in gene structure that are used for adaptive tuning of gene expression levels.

520A Mitonuclear Epistasis Modifies Gene Expression in *Drosophila* *James Mossman*[1], Leann Biancani[1], Zhijin Wu[2], David Rand[1]. 1) Ecology and Evolutionary Biology, Brown University, Providence, RI; 2) Department of Biostatistics, Brown University, Providence, RI.

Efficient mitochondrial function requires coordinated expression of both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). Mitochondria therefore provide an important model of gene-gene coevolution and interaction (epistasis). Here we describe a genetic system to investigate mitonuclear epistasis (G x G) in the Drosophila Genetic Reference Panel (DGRP). Our primary hypothesis postulates that mtDNA evolutionary divergence correlates with divergence in phenotypic values. Using a phenotypically-informed subset of a larger mitonuclear genetic panel, we focused on eight mitonuclear genotypes from two DGRP backgrounds. Each of two DGRP lines was introgressed onto two different *D. melanogaster* mtDNA haplotypes individually, or two different *D. simulans* mtDNA haplotypes individually; providing a mtDNA phylogenetic framework for comparison. We assayed gene expression using RNAseq on whole flies in both sexes individually to test the prediction that species-level mtDNA differences were associated with higher numbers of differentially expressed genes than individual haplotype-level genetic variants. We found large sex-differences in gene expression and a large influence of nuclear DNA background across both sexes. MtDNA haplotype modified gene expression and this effect was evident in both species-level and haplotype-level analyses. Moreover, effects of mtDNA variation were contingent on the nuclear background, evidencing mitonuclear epistasis for gene expression. Species-level and individual haplotype-level analyses provided gene sets with sometimes non-overlapping elements. We are now extending this mitonuclear framework to understand how mitonuclear epistases are influenced by diet in a wider context of gene x gene x environment interactions (G x G x E).

521B Epistasis in quantitative traits controlled by sexual differentiation in *Drosophila melanogaster* *Daniel W. McNelis*[1], Joyce Y. Kao[1], Ben E. Goulet[2], Jia Shen[3], Mark L Siegal[1]. 1) New York University, New York, NY; 2) Harvard University, Cambridge, MA; 3) Stony Brook University, Stony Brook, NY.

Despite advances in genomic technologies, the role of epistasis in the genetic architecture of quantitative traits

remains unclear. One manifestation of epistasis that has attracted attention is cryptic genetic variation (CGV), which comprises variants that have minimal effects except in the context of another mutation. CGV has been proposed to be a source of adaptive variation when selective pressures change and to be relevant to risk for complex diseases, such as type II diabetes and asthma. In this study, we examined variation in quantitative traits that are controlled by the sexual-differentiation pathway of *Drosophila melanogaster*. We used the *Drosophila melanogaster* Genetic Reference Panel (DGRP), a genome-sequenced collection of 205 naturally derived inbred strains, as our source of natural genetic variation. We deleted *intersex*, which encodes a subunit of the Mediator complex that is required for female development, from individual strains of the DGRP. We then quantified variation across strains by measuring trait values in homozygous mutants as well as heterozygous controls. Using mixed linear modeling, we detected a significant interaction between *intersex* mutational status and strain background on phenotypic outcomes, indicating that cryptic genetic variation exists for these traits. The interaction effect is much smaller than the main effect of removing *intersex*, yet it is comparable to the main effect of strain background. Going forward, it will be informative to investigate the revealed variation in more detail, leveraging the genome-sequence data from the DGRP strains to perform genome-wide association and to analyze genome-wide gene expression data to explore the molecular basis of CGV.

522C Genomic Regulation of Limited Lifespan and Reproductive Senescence *Grace Parker*, Trudy Mackay. North Carolina State University, Raleigh, NC.

Limited lifespan and senescence are near-universal phenomena. These quantitative traits exhibit variation in natural populations due to the segregation of many interacting loci and from environmental effects. Due to the complexity of the genetic control of lifespan and senescence, our understanding of the genetic basis of variation in these traits is incomplete. Our goal is to identify causal genes associated with increased lifespan and postponed reproductive senescence in *Drosophila melanogaster* by functional analyses of genetically divergent genes between five long-lived (O) lines selected for postponed reproductive senescence and five unselected (B) lines. Preliminary data assessing productivity of the reciprocal crosses of the O and B lines suggest that genes influencing reproductive senescence are maternally-controlled. Therefore, all of the candidate genes tested are expressed in the ovaries of females. To determine which of these candidate genes exhibit a quantitative change in lifespan or reproductive productivity, we assessed lifetime reproduction of candidate genes from the Vienna collection of RNAi lines in which gene expression is knocked down weakly throughout the body. We identified genes that limit and genes that extend longevity and are required for proper reproductive function. Identifying evolutionarily conserved genes affecting increased lifespan and delayed reproductive senescence is the first step towards understanding the evolutionary forces that maintain segregating variation at these loci in nature and may provide potential targets for therapeutic intervention to delay senescence in populations with increasing lifespans.

523A Population genomics of incipient speciation in the *Drosophila athabasca* species complex *Ryan Bracewell*, Karen Wong-Miller, Doris Bachtrog. Integrative Biology, UC Berkeley, Berkeley, CA.

To understand the genomic changes that facilitate speciation we need to study systems in the earliest stages of differentiation. The *Drosophila athabasca* species complex consists of three recently diverged semispecies that exhibit strong behavioral isolation, yet no morphological differentiation or postzygotic isolation. Female flies prefer males that sing the correct semispecies-specific courtship songs. In order to characterize genome evolution during speciation and to identify the genomic regions responsible for behavioral isolation, we first assembled a reference genome using long read technology (PacBio). The new *D. athabasca* assembly is of exceptional quality and is comprised of only 267 contigs with an N50 of 15 Mb and a total assembly length of 198 Mb. The assembly's gene completeness scores (BUSCO) rival that of the *D. melanogaster* assembly. Using this high-quality assembly as a backbone, we then used whole genome resequencing of 300 individuals from 16 locations across the United States to characterize patterns of genetic diversity and divergence. Our population genomic analyses show that within this recently formed complex, the three semispecies are indeed very recently diverged, yet genetically distinct. We also found little evidence of introgression between semispecies, even in areas of sympatry. Further, our genome scans identified highly divergent candidate regions in each semispecies that may harbor genes important during speciation. Our results provide a glimpse of genome evolution and divergence during incipient speciation and highlight how genome scans can be used to identify candidate genes responsible for reproductive isolation.

524B The molecular basis of hybrid sterility in two subspecies of *Drosophila pseudoobscura* *Nora Brown*, Chris Large, Nitin Phadnis. University of Utah, Salt Lake City, UT.

The evolution of reproductive isolating barriers, such as hybrid sterility and inviability, is a key step in the formation of new species. Despite decades of study, only a handful of incompatibility genes have been identified, though we know almost nothing of their molecular functions nor the mechanisms by which they cause hybrid incompatibilities. The Bogota and USA subspecies of *Drosophila pseudoobscura* are paradigmatic of the earliest stages of speciation, and thus provide a powerful system to investigate these long-standing questions. Our previous

genetic mapping approach identified the Bogota allele of the gene *Overdrive (Ovd)* is necessary for sterility in F1 hybrid males.

We used a systematic cell biological approach to investigate the molecular mechanisms of hybrid sterility in *D. pseudoobscura* hybrid males. Our findings show that the primary fertility defect is caused by a major misregulation of histone modifications during and immediately following meiosis II in spermatogenesis. This misregulation causes improper compaction of chromatin in post-meiotic spermatid nuclei, leading to a stall in spermatid development and, ultimately, sterility.

To investigate the molecular role of *Ovd*, we employed a CRISPR/Cas-9 based approach to natively tag *Ovd* with GFP in *D. melanogaster.* Our live imaging approach shows that *Ovd* has a strong cell cycle-dependent localization pattern, similar to other cell cycle checkpoint genes. We observe this pattern in embryonic syncytial divisions as well as meiotic divisions within testes, suggesting a cell cycle-dependent role for *Ovd* in both somatic and germline tissue. Together with the molecular data from F1 hybrids, these results suggest that *Ovd* may regulate chromatin dynamics during cell division. Our results provide initial insights into the molecular mechanism of *Ovd*-mediated hybrid sterility, and suggest for the first time a role for germline chromatin regulation in the evolution of reproductive isolating barriers between species.

525C The Ever Evolving Hybrid Incompatibilty of *D. melanogaster* and the *D. simulans* Clade *Jacob Cooper*, Nitin Phadnis. Biology, University of Utah, Salt Lake City, UT.

Hybrid incompatibilities are differences between the developmental plan of two species that manifest as extreme phenotypes. Interactions between just a few divergent genes can prevent even closely related species from forming reproductive progeny. By identifying and studying hybrid incompatibility genes, we can understand how the forces of evolution keep two species distinct. We have identified a new hybrid incompatibility gene that is required for hybrid F1 male lethality in the cross of *D. melanogaster* females to *D. simulans* males. Via a sequencing-based mutagenesis screen, we identified the *D. simulans* allele of *GST-containing FLYWCH zinc-finger protein* (*gfzf*) as a gene required for F1 male inviability. We demonstrated that knockdown of *D. simulans* allele of *gfzf* (*gfzf ^{sim}*) rescues hybrid F1 male inviability. As mutations in *gfzf ^{mel}* do not rescue hybrid males, only *gfzf ^{sim}* acts in a dominant manner to cause hybrid F1 male inviability. We used this method of hybrid male rescue to conduct the first test of a uniform genetic architecture for hybrid incompatibilities between the *D. simulans* clade and *D. melanogaster*. *D. simulans* is part of a clade of three species (*D. simulans, D. mauritiana,* and *D. sechellia*). Hybrids formed from each species with *D. melanogaster* show the same patterns of F1 hybrid incompatibilities. We predicted that knockdown of *gfzf ^{mau} and gfzf ^{sec}* should rescue hybrid males of *D. melanogaster* and their respective species, as we observed with *D. simulans*. Surprisingly, we find that knockdown of *gfzf* in crosses with *D. mauritiana* rescues hybrid males with greater efficacy than in crosses with *D. simulans*, whereas knockdown in crosses with *D. sechellia* show no rescue at all. We posit that the genetic architecture of hybrid F1 male inviability with *D. melanogaster* differes between all members of the *D. simulans* clade. Via polytene analysis, we find that *gfzf* is a telomere-associated factor that binds telomeres differentially between *D. melanogaster* and the species of the *D. simulans* clade. Our findings support the hypothesis that the architecture of hybrid incompatibilities with an outgroup species can continue to evolve even after clade radiation. Additionally, we find evidence supporting the hypothesis that interactions between DNA binding proteins and rapidly-evolving DNA sequences may play a role in speciation.

526A The Demographic History of the Drosophila nasuta Subgroup and Contributors to Reproductive Isolation *Dat Mai*, Matt Nalley, Doris Bachtrog. Integrative Biology, University of California Berkeley, Berkeley, CA.

The *Drosophila nasuta* subgroup is comprised of 14 species of different ages living in sympatry and parapatry throughout Southeast Asia. No naturally occurring hybrids have been discovered, yet many species pairs are interfertile in a laboratory setting. To infer the speciation history of this clade, we sequenced multiple strains of each species of the *D. nasuta* subgroup. Principal Component and phylogenetic analyses show that most species show distinct genetic clustering, but low levels of overall differentiation, consistent with recent divergence of species in this group.

We also explored the genomic landscape of hundreds of interspecies hybrids from this subgroup throughout multiple generations by inferring the ancestry of each hybrid individual along the genome with a Hidden Markov Model. We identified unlinked regions in high linkage disequilibrium, which are potential contributors to reproductive isolation between species. The study provides an opportunity to find loci that contribute to reproductive isolation between species within a clade, evaluate whether the same loci are incompatible between different species pairs, and allows us to estimate the rate at which reproductive isolation accumulates over time.

527B ***Drosophila melanogaster*** **is associated with distinct yeast communities within winery environments** *Allison Quan*, Michael Eisen. Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Though often studied independently in the lab, *Drosophila* and yeast participate in a natural mutualism. Fruit fly larvae depend on yeast as a nutrition source and adult flies vector yeast to new substrates. For wineries that use spontaneous fermentations, *Drosophila* act as important dispersal vectors of wine yeasts. Previously, we found that wild *Drosophila melanogaster* lines prefer specific lab strains of *Saccharomyces cerevisiae* and are able to distinguish between the volatiles produced by these yeast strains. However, little work has been done to characterize the specificity of this relationship in an ecological setting.

To characterize the spatial and temporal dynamics of this relationship in nature, we conducted a fungal amplicon study of the fungi vectored by *Drosophila* in two organic, Northern California vineyards. Contrary to what is known about yeasts on wine grapes, we found that the fungal communities vectored by flies are not distinct between vineyards. Instead, the fungal communities carried by flies group by vineyard area. Because the ability to locate yeasts directly affects *Drosophila* fecundity, we also measured the effects of natural yeasts on fly olfactory behavior, oviposition choice and larval development.

528C **Genome-wide association study on the behavioral isolation in** ***Drosophila melanogaster*** Juan Li[1,3], Hsi Chen[2], *Chau-Ti Ting*[2,3,4], Shu Fang[5]. 1) Beijing Institute of Genomics, Chinese Academy of Sciences; 2) Department of Life Science, National Taiwan University; 3) Institute of Ecology and Evolutionary Biology, National Taiwan University; 4) Genome and Systems Biology Degree Program, Research Center for Development Biology & Regenerative Medicine, National Taiwan University; 5) Biodiversity Research Center, Academia Sinica.

Sexual isolation among different populations is regarded as a process of incipient speciation. Previous studies found that sexual isolation existed between Zimbabwe (Z race) and other continental (M race) populations of *Drosophila melanogaster*. When given a choice, Z-race females preferred to mate with Z-race males, whereas M-race females mate with males of both races with little discrimination. To investigate the geographic distribution of Z-race, we examined the female preference between Z30 and FR males by multiple-choice cage experiments. The results showed an extensive polymorphism on both female preference and male mating success across all populations. As the initial discovery in Zimbabwe, most (94% = 116/124) of lines displayed strong to intermediate Z-female preference and only a few lines show little preference between Z30 and FR males. Since the female preference is a typical quantitative trait, we conducted a genome-wide association study to detect candidate SNPs responsible for the Z-female preference. Most of these candidate SNPs were located at non-coding regions, suggesting the behavioral divergence is mainly resulted from the change in gene expression. The linkage disequilibrium revealed at three genomic regions associated with the preference. This study provides the first detailed genomic analysis on the behavioral isolation in *D. melanogaster.*

529A **Deficiency mapping of female mate discrimination factors in** ***Drosophila sechellia*** *Masatoshi Tomaru*. Drosophila Genomics and Genetic Resources, Kyoto Institute of Technology, Kyoto, Japan.

Females of *Drosophila sechellia* rarely accept *D. melanogaster* males, whereas *D. melanogaster* females accept *D. sechellia* males fairly well. Since hybrid females accept *D. melanogaster* males better than *D. sechellia* females do, the discrimination by *D. sechellia* females against *D. melanogaster* males seems to be (partly) recessive. To explore factors responsible for female discrimination in *D. sechellia*, 84 strains of the third chromosome deficiency kit of the DrosDel lines were used for deficiency mapping. Since no or only a small number of hemizygous females were obtained from 20 deficiency strains, the other 64 deficiencies were used for mapping. These 64 deficiencies cover about 55% of the third chromosome. Behaviors of single pairs of a hemizygous hybrid female and a wild-type *D. melanogaster* male were observed. Overall, courtship was observed in 62.6% of the pairs and 56.2% of the courting males attempted to copulate. Successful copulation was observed in 38.0% of such pairs. Females hemizygous for one of seven chromosomal regions copulated with *D. melanogaster* males at 10% or less. These chromosomal regions in *D. sechellia* are candidates involving female discrimination factors.

530B **Gene Expression Profiling in the Larval Fat Body of Desiccation Selected** ***Drosophila melanogaster*** *Adriana Charles*, Eran Gefen, AJ Marlon, Allen Gibbs, Philippos Tsourkas. University of Nevada Las Vegas, Las Vegas, NV.

D. melanogaster subjected to desiccation (no food or water) display slower development and a higher body mass compared to fed controls due to an extended third larval instar. We hypothesize that desiccation selected will have a different gene expression profile compared to fed controls. *D. melanogaster* were subjected to desiccation (no food or water), starvation (no food only) for several generations. mRNA from the larval fat body was collected at 88 hours, 96 hours, 112 hours and 120 hours post-hatching for desiccation selected, starvation selected, and fed controls. Four replicate populations were used for each condition and time point. Gene expression was measured using two color cDNA microarrays. We analyzed the microarray data using a two way factorial design ANOVA implemented in R using a significance level of 0.05 with FDR correction. The FDR adjusted results showed 43 genes

differentially expressed for selection condition, 4834 genes for time, and 126 for interaction. We then used David 6.7 to explore which biological pathways are over represented for these genes. One biological process was shown to be over represented for the selection genes, ten for time, and seven for interaction. These over represented processes suggest desiccation selected and control populations are indeed developing differently.

531C Physiological and genomic effects of experimental evolution for desiccation resistance on *Drosophila melanogaster*
J. C. Kim[1], H. Ro[1], P. Shahrestani[1,2], M. R. Rose[2], J. Kezos[2], A. Talbott[1], L. D. Mueller[2], G. Rutledge[2]. 1) California State University, Fullerton, Fullerton, CA; 2) University of California, Irvine, Irvine, CA.

We examined five replicate populations of *Drosophila melanogaster* selected for enhanced desiccation resistance and compared them to five replicate control populations. The hypothesis tested here is that evolution for increased desiccation resistance results in changes in allele frequencies in the *D. melanogaster* genome, which in turn result in various correlated physiological and life-history effects. Observed characteristics include longevity, development time, heart function, and fungal resistance. Mortality assays were performed to compare the longevities between the desiccation resistant and control populations. The desiccation resistant populations lived longer compared to their controls. Flies from each population were subjected to induced cardiac arrest and monitored for recovery in order to examine the potential effects of desiccation selection on cardiac performance, but no significant changes in heart function were detected. Development time assays will be completed to observe the speed of maturation in these populations. Defense against fungal pathogens will be measured in these populations as survival post-inoculation with the fungal pathogen *Beauveria bassiana*. Whole genome sequencing of the desiccation resistant and control populations is currently underway. Although the specific analyses of the population genomes are yet to be performed, we expect that fixation of alleles has occurred in the desiccation resistant populations, because these populations have not reverted back to their original phenotypes after years of relaxed selection, and because desiccation resistance has a strong influence on defecation, lipid distribution, and other principal biological processes.

532A Coordination of resource availability with allocation in a natural population: lifespan and its heritability across dietary regimes
Enoch Ng'oma, Perinchery Anna, Stanley Patrick, King Elizabeth. Division of Biological Sciences, University of Missouri, Columbia, MO.

All organisms use nutrients to produce body tissues and maintain function over their lifespan. How organisms manage resources in varying availability over time is a central question in natural history theory. Sizable progress has been made in past years to unravel genetic and intermediate mechanisms that underlie biological evolution. However, most of these mechanisms, largely characterized in mutant laboratory populations, fail to be corroborated in natural populations. Using an eight-way multiparent population, we undertake experimental evolution of resource allocation strategies in diverse nutritional conditions designed to mimic selective pressures experienced in the wild. Here we present data on the heritability of lifespan measured using a half-sibling design of flies fed on high sugar, low protein, and control diets in the base population. In addition, we show how the response of lifespan to diet has changed after five generations of selection and discuss our expectations for the response to selection in future generations.

533B Patterns of physiological decline due to age and selection in Drosophila melanogaster
Parvin Shahrestani[1], Julian Wilson[1], Laurence Mueller[2], Michael Rose[2]. 1) Biological Science, California State University Fullerton, Fullerton, CA; 2) Ecology and Evolutionary Biology, University of California, Irvine, CA.

In outbred sexually reproducing populations, age-specific mortality rates reach a plateau in late life following the exponential increase in mortality rates that marks aging. Little is known about what happens to physiology when cohorts transition from aging to late life. We measured age-specific values for starvation resistance, desiccation resistance, time-in-motion and geotaxis in ten Drosophila melanogaster populations: five populations selected for rapid development and five control populations. Adulthood was divided into two stages, the aging phase and the late-life phase according to demographic data. Consistent with previous studies, we found that populations selected for rapid development entered the late-life phase at an earlier age than the controls. Age-specific rates of change for all physiological phenotypes showed differences between the aging phase and the late-life phase. This result suggests that late life is physiologically distinct from aging. The ages of transitions in physiological characteristics from aging to late life statistically match the age at which the demographic transition from aging to late life occurs, in all cases but one. These experimental results support evolutionary theories of late life that depend on patterns of decline and stabilization in the forces of natural selection.

534C Reassessment of linkage disequilibrium patterns in *Drosophila* three decades later
F. Uno, L.B. Klaczko. DGEB, University of Campinas/UNICAMP, Campinas, Brazil.

Drosophila mediopunctata harbors a rich polymorphism for paracentric inversions on the second chromosome, and seasonal and altitudinal patterns of chromosomal arrangement frequencies strongly suggests that natural selection operates on this system. Our primary object of study – the second chromosome – can be simplified into two main

regions that almost do not overlap: proximal and distal. There are nine inversions in the proximal region (PA0, PB0, PC0, *etc*) and eight in the distal (DA, DI, DS, *etc*). At first sight, recombination between distal and proximal inversions can occur and, in principle, 72 haplotypes can be formed (DA-PA0; DI-PB0; *etc*). Nevertheless, intense linkage disequilibrium between distal and proximal inversions was found in a natural population at Parque Nacional do Itatiaia (Rio de Janeiro State, Brazil) during the late 80's. Exploratory analyses revealed typical patterns of selection for the haplotypes sharing inversions DA, PA0, DP, DS, PC0, DI, PB0 (with the haplotypes DA-PA0, DP-PC0, DS-PC0 and DI-PB0 being favorably selected). In this work our aim is to assess if the linkage disequilibrium observed between haplotypes on the polymorphic chromosomal inversion system of *D. mediopunctata* might be maintained by natural selection. For that purpose, we analyzed 144 wild males of *D. mediopunctata* from three locations at the Parque Nacional do Itatiaia between 2015 and 2016. We estimated the inversion frequencies using the adult male sample method. We applied the linkage disequilibrium pattern analysis method to our more recent data and analyzed how this population evolved after 30 years. Given that in the absence of selection, linkage disequilibrium decays as a function of recombination between *loci*, we would expect it to fall to 41% of its original value due to recombination (r=0.017, t=320). Yet, for the most common haplotypes (DA-PA0, DI-PB0, DS-PCO and DP-PC0), the observed linkage disequilibrium values were very similar to those observed in the late 80's and differed significantly from what would be expected in a neutral model (X^2=96.4, d.f.=3, p<0.001). For these haplotypes, a relatively low selective coefficient would suffice to maintain the non-random association between distal and proximal inversions (s=0.144, DA-PA0; s=0.024, DI-PB0; and s=0.0276, haplotypes sharing inversion PC0). Our results lend further support to the action of natural selection on this chromosome inversion polymorphism. In addition, even though the data presented in this study are preliminary, they lend some support to a coadaptation model. Further studies may help elucidate whether these haplotypes are being selected as supergenes. Funding Agencies: CAPES, CNPq, FAEPEX-UNICAMP, FAPESP.

535A Characterization of Genetic Basis of Adult Reproductive Diapause in *Drosophila melanogaster*. *Sreesankar Easwaran*, Dominique Houston, Denise Montell. Molecular Cellular and Developmental Biology, University of California, Santa Barbara, CA.

Diapause is an animal behavior which delays or arrests its development and or aging in response to regularly recurring periods of adverse environmental conditions. Different insects undergo diapause at different points in the lifecycle. *Drosophila* adult females arrest their reproductive development by arresting ovary development at low temperature and short day. Diapause is a complex trait modified by multiple fitness-related traits and thus multiple genes are likely to be involved. Though the hormonal control leading to diapause variations have been characterized to some extent, the genetic components influencing this trait are poorly understood. Diapause has important roles in the tremendous success of the class Insecta, and this trait has been used in industries like silk industry to better preserve silkworm embryos or eggs.

Knowing its importance in insect lifecycle and the recent availability of the Drosophila Genome Reference Panel (DGRP) lines, we are investigating the genetic basis of *Drosophila* diapause using a genome-wide association study (GWAS). Approximately 200 inbred lines generated from a single outbred population of drosophila collected near Raleigh, NC comprise the DGRP. The availability of their sequences makes it a good resource for population genomics and association mapping.

We induce diapause by keeping these lines at low temperature (10°C) and short day length (8L:16D) for 5 weeks, and then scoring the propensity to diapause using diapause related traits like lifespan extension, fertility, and fecundity. We see a good variation in these traits among the DGRP fly lines, and we are in the process of undertaking the GWAS analysis. We are also characterizing the molecular and cellular basis of the anatomical changes that occur in the fly ovary during diapause. The goal of this study is to obtain general insights into the genes, cell types, and molecular pathways involved in initiating and maintaining insect diapause programs with the ultimate goal of enhancing fly stock keeping techniques and revealing opportunities for control of insect populations.

536B The hybrid swarm: whole genome association studies from ultra-shallow sequencing of a large, randomly outbred population *Cory A. Weller*[1], Subjhash Rajpurohit[2], Susanne Tilk[3], Dmitri Petrov[3], Paul Schmidt[2], Alan O. Bergland[1,3]. 1) Department of Biology, University of Virginia, Charlottesville, VA; 2) Department of Biology, University of Pennsylvania, Philadelphia, PA; 3) Department of Biology, Stanford University, Stanford, CA.

Reference panels comprised of inbred lines are often utilized in genetic mapping studies to uncover genetic associations with phenotypic variation, providing insight into how genotype is translated to phenotype. While mapping can be performed directly on these reference panels, an outbred multi-parent population generated by crossing these inbred lines can offer increased mapping resolution. Despite the advantage of outcrossed multi-parent populations, association studies have classically utilized a limited number of founding haplotypes due to computational limitations. Here, we apply a low-cost method that leverages the genetic diversity of a large reference panel, the *Drosophila* Genetic Reference Panel (DGRP). We generated a large panmictic mapping population, which we refer to as a hybrid swarm, by randomly outbreeding nearly 200 inbred lines from the DGRP over four generations.

Using a modified imputation technique, we were able to rapidly and accurately recover whole genomes from ultra-low coverage sequencing data (observing as few as 5% of polymorphisms genome-wide) and map associations for starvation tolerance with high accuracy and specificity. We additionally describe a pipeline for rapid simulation of *in silico* association mapping and compare the power and accuracy of various potential experimental designs that incorporate different numbers of founding haplotypes. The simulation pipeline incorporates user-defined parameters to generate a synthetic hybrid swarm mapping population, readily accepts any sequenced reference panel, and calculates association likelihoods for any defined pattern of genetic architecture (e.g. number of causative loci, allele frequency, and degree of variation explained). Taken together, these tools provide a new, resource-efficient approach to fine-scale genetic mapping.

537C Adaptation of Baculovirus via recurrent positive selection and gene turnover *Thomas Hill*, Robert Unckless. Molecular Biosciences, University of Kansas, Lawrence, KS.

Previous work has found that immune system genes and the parasites they regulate often evolve rapidly, in a molecular arms race with each other. This is especially true for RNA viruses and the genes in the siRNA pathway, a complex of genes which uses virus complementary small RNAs to suppress their activity. Though the molecular evolution of RNA viruses and their hosts have been extensively studied, the more complex DNA viruses have been largely ignored. DNA viruses, including baculoviruses & nudiviruses, are an emerging insect pathogen with practical applications in population control and understanding them through an evolutionary lens is necessary to properly utilize them. To better understand how these viruses evolve alongside their host system, and to inform us on future work studying a *Drosophila* nudivirus, we look for signatures of selection across different baculovirus & nudivirus genomes and within a population of one baculovirus. We find primarily strong purifying selection acting across the genomes among baculoviruses, however we find several genes associated with envelopes and capsids are rapidly evolving, as expected to escape the host immune system. Interestingly, we also find that genes associated with viral replication and that complex with RNA polymerase are under recurrent positive selection, suggesting these genes are a common target for suppression in the host.

538A Temperature influence on the altitudinal distribution of two sibling species of *Drosophila* from the Neotropical *tripunctata* group *Marcos Batista*, Felipe Rocha, Louis Klaczko. Genetica, Evolução e Bioagentes, UNICAMP, Campinas, SP, Brazil, São Paulo, Brazil.

Variation of ecophysiological traits may help to explain geographic distribution patterns of *Drosophila* sibling species. Many traits in ectotherms have optimal performance within specific temperature ranges. Elevational gradients are potentially informative for characterizing differences of sibling species distributions. We collected two sibling species of the *tripunctata* group – *Drosophila mediopunctata* (*MPT*) and *D. unipunctata* (*UNI*) – at four altitudes (745, 808, 955 and 1073m) located at a continuous Atlantic Rainforest reserve in two consecutive years (2009-2011), with two collections at the hot-rainy season and two at the cold-dry season. Their distributions showed a nearly constant displacement, with *MPT* always occupying higher altitudes than *UNI* (*MPT*: cold-dry mean altitude of collection [CMAC]: 915 m; *UNI* [CMAC]: 790 m; *MPT* hot-rainy mean altitude of collection [HMAC]: 999 m; *UNI* [HMAC]: 891 m). Both species and season showed strong and significant statistical differences ($F = 30.64$; df: 1; $p = 0.00000$; $F = 19.48$; d.f.=1; $p < 0.00005$). We characterized the thermal range of fertility, an important fitness component, for each species and found evidence for differential thermal adaptation: Maximum fertility observed for *MPT* (90.7%) was set at 16ºC, while it was set at 18ºC for *UNI* (93.3%). Inferior limit was fixed at 12ºC (40% of fertile males) for *MPT* and 30% for *UNI*; while superior limit was fixed at 25ºC for *MPT* with 2.8% of fertile males, while *UNI* fixed at 28ºC (2.8% of fertile males). Our results suggest that the altitudinal distribution displacement results from differential thermal adaptation: *MPT* seems to be adapted to lower temperatures and occupies higher altitudes, while *UNI* tolerates higher temperatures and occurs at lower altitudes. However, their thermal ranges overlap at most temperatures, suggesting that additional variables (e.g. habitat choice, competition, differential survival etc.) may also play a role to determine their distribution in the field.

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539B The genetic and developmental basis of rapid trait evolution between *Drosophila* species *J. F. D. Hagen*, A. Blogg, K. Tanaka, A. P. McGregor, M. D. Santos-Nunes. Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom.

In this study, we identify loci which explain a phenomenon of rapid phenotypic trait evolution.

Although *Drosophila simulans* and *Drosophila mauritiana* diverged only 240 thousand years ago, they already show striking differences in the morphology of the male genitalia. The rapid evolution of male genital between these species reflects a more general pattern among most animal species of internal fertilisation.

The claspers, which are structures that seem to be important for the correct positioning and attachment of the male during copulation, are twice as large and carry a third more sensory bristles in *D. mauritiana* than in *D. simulans.* In

addition, *D. mauritiana* has smaller posterior lobes and larger anal plates than *D. simulans*.

To unravel how this rapid morphological evolution has occurred, we performed QTL and high-resolution introgression mapping on clasper morphology. This analysis showed that the genes underlying clasper size divergence between *D. mauritiana* and *D. simulans* are located in two small candidate regions on the left arm of the 3rd chromosome. During this mapping, we could also identify two additional regions which contain genes contributing to posterior lobe size divergence between the two species. Our results suggest that there are no pleiotropic effects between the genes underlying clasper and posterior lobe size divergence, and that each show differential species dominance, indicating separate evolutionary histories. Although generally these loci affect each trait in the same direction and act additively, we have also found evidence for epistasis.

An RNAi screen in *Drosophila melanogaster* of all genes in one candidate region for clasper divergence has shown that only three are required for genitalia development – *Grunge*, *fear of intimacy* and *Cpr66D*. We are currently using CRISPR/Cas9 to generate reciprocal hemizygotes of the cis and trans acting elements of these three candidate genes. This will allow us to identify which loci underlies the rapid evolution of genital morphology between *D. simulans* and *D. mauritiana*, and illustrate if this is achieved by changes in coding or non-coding elements.

This study will also allow us to test the fitness consequences of rapid morphological diversification, and illustrate the effect of single locus evolution on reproductive isolation between closely related species.

540C The developmental transcriptome of *Musca domestica* embryos reveals the origin of new elements of Dpp gene regulatory network in *D. melanogaster.* *Christian Hodar*[1,2], Veronica Cambiazo[1,2]. 1) University of Chile - INTA, Santiago, Chile; 2) Fondap Center for Genome Regulation (CGR).

Introduction. The dorsal-ventral axis specification network (DVN) in dipterans represents a great model to understand the evolution of development. Analysis of gene expression of orthologs genes of the DVN between *A. gambiae*, *M. abdita* and *D. melanogaster*, indicate that dorso-ventral morphological variations, including the transition from two extraembryonic epithelium and the formation of an unique dorsal membrane in higher cyclorraphan flies, amnioserosa (AS), were consequence of expression changes of genes that command DV patterning. Since the origin of amnioserosa has been estimated occurs ~120Mya and the radiation of the species from Drosophila clade ~50Mya, we propose that *M. domestica*, diverged from *D. melanogaster* ~100Mya, will contribute to increase the knowledge about evolution of gene expression during development and in particular to elucidate whether the differences in dorsal patterning between *D. melanogaster* and *M. abdita* (or *A. gambiae*) correspond to changes that occur before the Drosophila genus radiation and after amnioserosa formation. We also propose that taxa specific genes can be evolve in high cyclorraphan flies evolution and recruited as new members of the dorso-ventral regulatory network in *D. melanogaster.*

Methods. We perform RNAseq of five developmental stages of M. domestica embryos and processed data was used in comparative bioinformatics analysis for reconstruction of DVN and for identification of new potential targets for this network in D. melanogaster. We use for in vivo transcript detection in situ hybridizations in whole-mount embryos (ISH).

Results and Conclusions. We identify in M. domestica several orthologs of the Drosophila DVN, expressed during embryo development and with this data we construct the regulatory network for dorso-ventral patterning. We examine the spatial expression of some genes in M. domestica in order to determinate if these patterns are retained among the three species. We also analyzed the expression of new potential members of DVN in D. melanogaster and examine its regulatory dependence of Dpp and Zen, the major regulators for dorsal-ventral specification in Drosophila species. Our results suggest that the transcriptome of M. domestica embryos during early development remains conserved in the higher Cyclorraphan lineages. In particular we prove that the expression of key genes for DVN are conserved between M. domestica and D. melanogaster. Also we prove that new evolved genes in Drosophila lineages are recruited into this network and are regulated by Dpp and Zen in dorsal domain of the embryo.

541A Evolutionary Dynamics of a Robust Developmental Trait: Segment Allometry across 12 *Drosophila* Species *Gizem Kalay*[1], Joel Atallah[2], Amanda E. Crofton[1], Austin M. Tang[1], Michael May[1], Hannah Maravelias[1], Mohan K. Murugesan[1], Susan E. Lott[1]. 1) The Department of Evolution and Ecology, University of California, Davis, Davis, CA; 2) Department of Biological Sciences, The University of New Orleans, New Orleans, LA.

To understand the genetic and environmental basis of phenotypic change, studies have often focused on rapidly evolving traits. However, many developmental traits most critical to the fitness of an organism are under high levels of developmental constraint and are robust to genetic and non-genetic perturbations. Do these traits change over evolutionary time? If so, do they change in small frequent steps or rare big jumps? Here, we use *Drosophila* larval segmentation as a model system to investigate the evolutionary dynamics of a developmentally constrained and robust trait. At the embryonic stage, segment allometry (position/size of segments relative to body length) has been

examined in a few species, and was found to be largely invariant within, but variable between species. In order to systematically and precisely quantify patterns of change in segment allometry, we developed a high throughput imaging and analysis pipeline. Using this pipeline we have extracted position information from 1st instar larvae, across 12 species spanning the *Drosophila* genus. We find considerable variation in the position of each segment between 12 *Drosophila* species that is not correlated to phylogenetic distance. Despite regulation by a complex interconnected network of genes, the positions of different segments change independently of one another. The magnitude of evolved changes in segment position is most striking in two species. Toward the posterior end of the larvae, *D. persimilis* and *D. mojavensis* have significant shifts to the posterior and anterior, respectively. This is at least partly due to the stark difference in the length of posterior spiracles in these species. Our data show that there are significant differences in this developmentally robust trait between even closely related species. Lack of strong phylogenetic signal suggests that larval segment allometry may evolve through large phenotypic changes on some lineages rather than through the continuous buildup of small phenotypic changes. Further studies will pursue the genetic causes underlying differences in segment allometry between species, as well as the impact of the environment on *Drosophila* segmentation.

542B Functional Evolution of a Morphogenetic Gradient *Chun Wai Kwan*, Jackie Gavin-Smyth, Edwin Ferguson, Urs Schmidt-Ott. University of Chicago, Chicago, IL.

Bone Morphogenetic Proteins (BMPs) pattern the dorsal-ventral axis of bilaterian embryos; however, their roles in the evolution of body plan are largely unknown. We examined their functional evolution in fly embryos. BMP signaling specifies two extraembryonic tissues, the serosa and amnion, in basal-branching flies such as *Megaselia abdita*, but only one, the amnioserosa, in *Drosophila melanogaster*. The BMP signaling dynamics are similar in both species until the beginning of gastrulation, when BMP signaling broadens and intensifies at the edge of the germ rudiment in Megaselia, while remaining static in Drosophila. Here we show that the differences in gradient dynamics and tissue specification result from evolutionary changes in the gene regulatory network that controls the activity of a positive feedback circuit on BMP signaling, involving the *tumor necrosis factor alpha* homolog *eiger*. These data illustrate an evolutionary mechanism by which spatiotemporal changes in morphogen gradients can guide tissue complexity.

543C Comparative approaches to reveal underlying constraints on enhancer sequence divergence in the *Drosophila* Montium clade *C. Martinez*[1], M. Bronski[1], A. Kopp[2,3], M. Turelli[2,3], M. Eisen[1,4,5]. 1) Dept. of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Dept. of Population Biology, UC Davis, Davis, CA; 3) Dept. of Evolution and Ecology, UC Davis, Davis, CA; 4) Dept. of Integrative Biology, UC Berkeley, Berkeley, CA; 5) Howard Hughes Medical Institute, University of California, Berkeley, CA.

Proper regulation of gene expression is often reliant on non-coding cis-regulatory genomic regions, especially enhancers. Astonishingly, enhancers can sustain substantial sequence reorganization between distantly related species while still maintaining regulatory function. Therefore monumental questions remain: how is this function maintained and what are the constraints acting on enhancer sequence divergence? To address these questions we have created a valuable resource for studying enhancer divergence by sequencing the genomes of 20 species from the *Drosophila* Montium clade. The rapid speciation of the Montium clade allows accurate alignment of non-coding regions to assess orthology and the ability to capture species-specific sequence divergence signals to test hypotheses on enhancer architectural evolution. Congruently we are testing species-specific enhancer function in Montium using the MS2-GFP reporter system, which allows the direct measurement of active transcription in live embryos and the assessment of subtleties in enhancer directed transcription. We have created 5 lines that express MS2-GFP driven by *Eve Stripe 2* (*ES2*) from four Montium (*Drosophila rufa, Drosophila bocki, Drosophila birchii,* and *Drosophila jambulina*) species and *Drosophila melanogastor* as control. Using confocal microscopy, we are measuring precise patterns of transcription localization, burst size, frequency, and duration. We will be presenting work that shows the extent of which *ES2* directed function is maintained between Montium species. The combination of comparative genomics and functional analysis in the Montium Clade provides an unprecedented glimpse into how enhancers evolve and regulate the spatiotemporal gene expression patterning needed to direct multicellular development.

544A Evolution of Bicoid involved epistatic interactions between two specific amino acids in its DNA-binding domain *Pinar Onal*[1], Rhea Datta[1], Qinwen Liu[2], Joe Thornton[2], Stephen Small[1]. 1) Biology, New York University, New York, NY; 2) Department of Human Genetics and Department of Ecology and Evolution, University of Chicago, IL.

How evolutionary changes affect developmental networks and the processes they control is a fundamental question in biology. Bicoid (Bcd) emerged after a gene duplication event in Hox3 locus together with paralogous protein Zerknullt (Zen) in Cyclorrhaphan flies. It has transformed into a key regulator of anterior patterning network in *Drosophila*, likely due to newly gained features, such as a shift in DNA-binding specificity in the homeodomain (HD), and the acquisition of the ability to bind to RNA. To directly test hypotheses about ancient mutations that

remodeled a transcriptional network driven by Bcd, we combined Ancestral Protein Reconstruction (APR) with engineering of transgenic animals and analysis of molecular developmental processes. We predicted the HD sequences of ancestor of Zen and Bcd and the diagnostic residues for Bcd's evolution using a maximum likelihood-based APR method. We first tested the binding specificities and gene regulatory functions of ancestral HDs, then investigated the impact of the diagnostic residues on the evolution of HD function. The ancestor of Zen and Bcd, AncZB, had a Zen-like binding specificity and did not activate any Bcd-dependent target genes in *Drosophila* embryos. Among 11 amino acids that were predicted to be diagnostic in Bcd HD evolution, Lysine residue at the HD position 50, K50, was sufficient to change the DNA-binding specificity of the AncZB HD *in vitro* and confer some of Bcd-like activities to the partially ancestral protein *in vivo*. Arginine 54 (R54), on the other hand, is required but not sufficient for RNA-Binding activity and is not detrimental for DNA-binding specificity of the HD. However, it acts epistatically with K50 and alters AncZB-HD's function significantly when introduced together with K50. Our results suggest that most of the patterning specificity of the Bcd protein lies in its HD, and that Bcd evolved from a Zen-like ancestor and gained novel functions by a single mutation that changed the specificity and step-by-step mutations that acted epistatically and altered the affinity of the HD towards target cis-regulatory sequences.

545B *Drosophila* pair-rule gene orthologs in the sequentially segmenting insect *Oncopeltus fasciatus* (Hemiptera: Lygaeidae). *Katie Reding*[1], Mengyao Chen[1], Yong Lu[2], Leslie Pick[1]. 1) University of Maryland, College Park, MD; 2) SUNY Stony Brook, Stony Brook, NY.

Studies of segmentation in *Drosophila melanogaster* have greatly contributed to our current understanding of embryonic patterning in insects. From these studies, a model for a developmental hierarchy has emerged, one level of which is represented by the pair-rule genes (PRGs) which encode transcription factors involved in promoting the formation of body segments. Studying this family of genes in other insects can broaden our knowledge of the evolution of the regulatory networks involved in early embryonic development. Interestingly, previous work showed that orthologs of the *Drosophila* PRG *eve* and a non-PRG *E75a* have non-PRG and PRG function, respectively, in the sequentially segmenting insect, *Oncopeltus fasciatus* (Liu & Kaufman, 2005; Erezyilmaz et al., 2009). We are thus evaluating the roles of the remaining *Drosophila* PRG orthologs in *O. fasciatus*. Searches in the *O. fasciatus* draft genome yielded partial sequences for most orthologs. RT-PCR was performed to determine time of expression, and whole mount *in situ* hybridization is underway to visualize expression patterns. So far, RT-PCR and *in situ* results suggest that *Of-odd, -prd, -run,* and *-slp* all play a role in segmentation, but likely do not function as PRGs in *O. fasciatus*. Expression of *Of-odd, -run,* and *–slp* all peak during the late blastoderm stage, when expression of genes involved in segmentation is expected. *In situ* hybridization reveals that both *Of-odd* and *Of-prd* are expressed in six stripes in the blastoderm, corresponding to the number of segments specified at this time. Simultaneous staining of *Of-odd* and *Of-E75a*, a known pair-rule gene in *O. fasciatus*, shows that *E75a* is expressed between every two *Of-odd* stripes, suggesting that *Of-odd* is a segment polarity gene in this species. *Of-ftz-f1*, *-ftz,* and *-h* are expressed during, but also after, the late blastoderm stage, and expression of *Of-opa* is only marginal. Overall, these results suggest extensive re-wiring of regulatory genes controlling segmentation in different insects.

546C Microhabitat preferences of some *Drosophila* species, including *Drosophila melanogaster* and *Drosophila simulans*, in southeastern Connecticut *Phillip Barnes*[1], Gabriel Chandler[2], Flora Drury[1], Christopher Krupenye[1], Patricia Arenson[1], Alex Ellison[1], David Aigler[1], Lindsey Dinsmore[1], Austin Meszaros[1], Katrina Salk[1]. 1) Dept Biol, Connecticut Col, New London, CT; 2) Dept Math, Pomona Col, Claremont, CA.

Microhabitat preference was studied in *Drosophila melanogaster*, *Drosophila simulans*, *Drosophila hydei*, *Drosophila immigrans*, and *Drosophila busckii* in southeastern Connecticut, USA. Two microhabitats were examined using compost bins, one of which is used year-round for household and garden waste containing vegetables, fruit, and houseplant materials, while the second bin is used in the fall, starting in late September or early October, for the pressings from red grapes (marc) for home wine production. Stereomicroscopy was used to identify species, and a multinomial logistic regression model was used to test for significant differences in the ratio of *D. melanogaster* to the other four species between bins and among dates. In 2009, the five species were all present in the vegetable bin prior to the introduction of the marc in the grape bin. In 2008 and 2009, after the grape marc was introduced into the grape bin, the proportion of *D. melanogaster* relative to each of the other four species was found to be significantly greater in the grape bin than in the vegetable bin. The proportion of each of the other four species, relative to *D. melanogaster*, was significantly greater in the vegetable bin than in the grape bin. In the grape bin, *D. melanogaster* was always the most frequent species by a large margin. In the vegetable bin, *D. melanogaster* was often less frequent than several of the other species: *D. hydei* and *D. immigrans* in 2008 and *D. simulans* and *D. immigrans* in 2009. However, the relative proportions of the four species compared to *D. melanogaster* varied significantly depending on the date. In particular, during the coldest sampling period (Oct. 22, 2009), *D. melanogaster* had become the most frequent species by a large margin in both the vegetable and grape bins.

547A Influence of the microbiota on life history adaptation in *Drosophila melanogaster* *Amber Wise*[1], Melinda L. Koyle[1], Paul S. Schmidt[2], John M. Chaston[1]. 1) Brigham Young University, Provo, UT; 2) University of Pennsylvania, Philadelphia, PA.

Life history tradeoffs are a fundamental aspect of organismal adaptation to spatially varying selection, with most current work focusing on how the environment selects an organism's genotype for optimal resource allocation in a given environment. For example, organisms that favor reproduction over somatic maintenance tend to have shorter development times to maturity and high early fecundity; whereas other strategies balance lower initial reproduction rates over a longer lifespan to increase fitness. In *Drosophila melanogaster*, a key model for understanding these life history tradeoffs, recent work has clearly demonstrated that associated microorganisms ('microbiota') can influence the magnitude of individual life history traits such as energy storage, development rate, and lifespan. Combining previously published and preliminary data we suggest that the microbiota not only contribute to, but also can be key players in local adaptation by showing that an isogenic fly line reared with different bacterial species displays life history strategies consistent with somatic maintenance or early reproduction depending on the genetic function of the associated species. We further use age-structured population growth models to reveal that *Acetobacter* species confer increased fitness on flies reared in the laboratory on a nutrient-rich diet. Finally, by surveying the microbial composition of wild-caught flies and measuring phenotypes of wild-caught fly populations associated with different microbial communities, we provide further support for a model where microbial identity and function plays a key role in local adaption.

548B Plasticity of expression and plasticity of phenotype: temperature-modulation of *ebony* expression may underlie the variation of dark spots on the abdomen of *Drosophila mediopunctata* *F.B. Rocha*[1], L.B. Klaczko[2]. 1) Departamento de Genética e Biologia Evolutiva, University of São Paulo - USP, São Paulo, Brazil; 2) DGEB, University of Campinas - UNICAMP, Campinas, Brazil.

Drosophila species of the *tripunctata* group have abdomens with overall yellowish pigmentation and up to three dark spots on the dorsal midline. *Drosophila mediopunctata* flies may have four phenotypes for this trait: 0,1,2 or 3 spots, varying in a posterior-anterior gradient from the A6 to the A4 tergites. Cuticle melanization in *Drosophila* results from the balance between the expression of *yellow, tan* (promote melanization) and *ebony* (inhibits melanization). Temperature inhibits melanization in *Drosophila*, and we found previously that in *D. mediopunctata* this yields a plastic response on spot number that is genetically variable. We assessed the patterns of phenotypic and gene expression variation at flies from three genotypes (two inbred strains [29I - zero spots; 229ET - three spots] and their F_1) raised at three temperatures – 15, 18 and 21°C – to investigate the developmental basis of this gene-environment interaction. We quantified the phenotype as the percentage of darkly-pigmented area of fully-developed adults and the expression of *yellow, tan* and *ebony* by the areas marked with RNA DIG-labelled probes in dissected abdomens of flies at late pupal stages. In all genotypes, flies raised at higher temperatures were more lightly-pigmented than flies raised at lower temperatures. In each temperature, 229ET flies had maximum pigmentation, 29I had minimum pigmentation and their F_1 had consistently intermediary phenotypic values. Both *ebony* and *yellow* showed consistent and significant ($p<0.05$) patterns of expression variation among genotypes, confirming previous results: heavily-pigmented flies had higher *yellow* expression and lower *ebony* expression. There was no evidence of association between *tan* expression and the pigmentation phenotype ($p=0.68$). *ebony* expression varied as a positive linear function of the developmental temperature ($R^2=0.153$; $p<0.005$) in all genotypes. These results support the hypothesis that phenotypic plasticity of *Drosophila* abdominal pigmentation in response to temperature results from changes in *ebony* expression: as the developmental temperature increases, *ebony* expression increases, therefore reducing the overall level of cuticle melanization. Furthermore, they suggest that the mechanism underlying spot number plasticity in *D. mediopunctata* is present and active even in genotypes that lack such response, pointing to additional factors to account for the lack of plasticity of extremely-pigmented genotypes. Funding Agencies: CAPES, CNPq, FAEPEX-UNICAMP, FAPESP.

549C Proteomics and Functional Genomics of the *D. grimshawii* Sperm and Associated Male Reproductive Tract Tissues *T. Karr*[1], J. Oses[2], A. Burlingame[2], B. Oliver[3]. 1) Drosophila Genomics and Genetic Resources, Kyoto Institute of Technology, Kyoto, Kyoto Prefecture, Japan; 2) NIH/NIGMS National Mass Spectrometry/Proteomics Center, UCSF, San Francisco, CA 94158-2517; 3) Laboratory of Cellular and Developmental Biology, NIDDK, NIH.

High throughput shotgun (bottom up) mass spectrometry has become a staple for the study of male reproduction in Drosophila and other diverse taxa. First applied to the Dmel, a growing list of sperm proteomes in mammals and other arthropods is opening up new areas of comparative and functional genomic studies not previously possible. However, very little is known about the proteomes of the associated reproductive tissues, and here we present a proteomic analysis of the D. grimshawii testis (TE), seminal vesicle (SV), accessory gland (AcG) and sperm (SP). These proteomes represent the first comprehensive study of these major components of the Drosophila male reproductive tract. Overall our study identified 4257 total UniProt IDs (1696 TE; 572 SV; 1084 AcG; 687 SP Flybase

genes). From this list we obtained 1989 orthologous Dmel IDs for network, genomic and functional analyses. Comparative molecular evolutionary analysis will also be presented demonstating apparent sub-functionalization of specific GO protein groups. This work establishes a framework for additional studies designed to provide deeper understanding of the evolution of male sexual reproduction traits.

550A Characterizing RNA transcripts from a "selfish" B chromosome in the jewel wasp *Nasonia vitripennis* *J. Aldrich*[1], C. Clifford[2], P. Ferree[1]. 1) W. M. Keck Science Department, The Claremont Colleges, Claremont, CA; 2) Pitzer College, Claremont, CA.

Insects of the order Hymenoptera (bees, wasps, ants, etc.) determine sex via a system of haplodiploidy—females develop from fertilized diploid eggs while males develop from unfertilized haploid eggs. In the jewel wasp *Nasonia vitripennis*, this system is disrupted in the presence of a nonessential B chromosome called PSR (Paternal Sex Ratio). This chromosome is only carried by males and is found in very low levels in natural populations. In order to ensure its own propagation, PSR severely biases the sex ratio by converting fertilized female-destined embryos into males. This occurs at the expense of the other paternally inherited chromosomes, all of which fail to resolve during the first round of division and are subsequently eliminated. Although paternal genome elimination is characterized by the disruption of a number of specific histone post-translational modifications, the mechanism underlying this phenomenon remains unknown. Recent work has identified several RNA transcripts specific to PSR-carrying males. Using a combination of PCR and cytological techniques, we have found that these transcripts map specifically to the PSR chromosome and are expressed throughout development, most notably in the testis. While their precise molecular functions and evolutionary origins remain unclear, preliminary evidence does suggest a potential role in mediating one or more aspects of PSR's biology.

551B *White* gene from *Aedes aegypti* mosquitoes is sex -linked and is involved in the toxicity of Cry4B toxin from *Bacillus thuringiensis* subsp. *israelensis* *Jianwu Chen*, Karlygash Aimanova, Sarjeet Gill. Department of Cell Biology and Neurosciences, University of California, Riverside, CA 92521.

The white protein belongs to an ATP binding cassette (ABC) transporter subfamily, ABCG. In *Drosophila melanogaster* it is known to be involved in the eye pigment precursor transport and in the regulation of lipid trafficking and pleiotropic drug resistance. In the lepidopteran diamondback moth, *Plutella xylostella,* down-regulation of the white gene is associated with Cry1Ac toxin resistance in. Much less is known of this gene in *Aedes* mosquitoes and its role in Cry protein toxicity. Here we investigated its role in *Aedes* mosquitoes. Hence, the *white* gene was knocked out using CRISPR-Cas9. Among adult mosquitoes derived from injected eggs, about 30% of them had mosaic eyes and their genotype was confirmed by T7 endonuclease assay. From crossing experiments, it was observed that the white gene was linked with a recessive female-determining factor (m) in the 1st chromosome and the approximate recombination ratio between white gene and female-determining factor (m) is 1.26%. Hence as in *Drosophila,* this gene is sex–linked. Sequencing of the genomic DNA of white-eye homozygous mutants showed the presence of a two nucleotides deletion in the ORF. Homozygous mutant larvae were then used in bioassays utilizing Cry toxins from *Bacillus thuringiensis* subsp. *israelensis* (Bti). These mutant larvae were more resistance to Cry4B toxin (> 4-fold) than wild-type larvae, but not to Cry11A toxin from the same bacterial strain. In sum, the female determining factor-linked white gene from *Aedes* mosquitoes confirms its conserved function in eye pigmentation. More interestingly, it is involved in Cry4B toxicity against *Aedes* mosquito larvae.

552C Butterfly eye evo-devo: stochastic patterning and expanded color vision *Michael Perry*[1], Michiyo Kinoshita[2], Giuseppe Saldi[1,3], Lucy Huo[1], Kentaro Arikawa[1], Claude Desplan[1,3]. 1) Department of Biology, New York University, New York, NY; 2) Department of Evolutionary Studies of Biosystems, SOKENDAI, Hayama, Japan; 3) New York University Abu Dhabi, Abu Dhabi, UAE.

Butterflies use color vision extensively to navigate the natural world. Most species express a broad range of color sensitive Rhodopsins in three stochastically distributed types of ommatidia ("unit eyes"). Their retinas are more complex than those of *Drosophila*, which contain only two stochastically distributed ommatidial types. We investigated how butterflies generate three stochastic types during development, resulting in a more diverse retinal mosaic that provides the basis for additional color comparisons and an expanded range of color vision.

Using genome sequencing, gene expression, and CRISPR gene knock out we show that the regulatory network that defines photoreceptor and ommatidial types in *Drosophila* is redeployed in butterflies (*Papilio xuthus* and *Vanessa cardui*) but is modified to generate additional types. In *Drosophila,* a stochastic decision of whether to express the transcription factor Spineless in R7 photoreceptors determines which of two types of ommatidia is specified. In butterflies, two R7s are recruited per ommatidium during development, providing the opportunity for two independent stochastic choices. CRISPR knock-out shows that *Spineless* controls stochastic patterning and all coordinated aspects of ommatidial type. This modification allowed for additional color comparisons in a more diverse retina, in turn allowing for the evolution and deployment of newly evolved Rhodopsins, including a red-sensitive Rhodopsin in *Papilio*. These efforts provide evidence that our extensive knowledge of patterning in

the *Drosophila* visual system applies to other groups, and that adaptation for specific visual requirements can occur through modification of this network.

553A The Conserved Pair-Rule Gene *odd-paired* Functions as Anterior Determinant in the Moth Fly *Clogmia albipunctata* Via Alternative Transcription *Y. Yoon*[1], J. Klomp[2], U. Schmidt-Ott[1]. 1) The University of Chicago, CHICAGO, IL; 2) University of Illinois at Chicago, CHICAGO, IL.

During early fly development, the anterior-to-posterior (AP) embryonic axis is initially specified by maternally localized axis determinants. Recently, it has been shown that structurally unrelated anterior determinants have evolved in different fly lineages. Examples are the *Hox-3* related gene *bicoid* in Drosophila and the *TCF/pangolin* related gene *panish* in the midge Chironomus. Both genes function as long-range pattern organizers and suppress posterior development but are absent in most other fly lineages (Klomp et al, 2015, *Science* 348, 1040-1043), implying that many flies independently evolved different anterior determinants. Our goal is to understand how unrelated long-range axis determinants evolve and whether they share common mechanisms of action. As part of this research program we asked how the moth fly *Clogmia albipunctata*, a basal-branching fly that lacks *bicoid* as well as *panish*, determines the AP polarity.

We compared the maternal transcript levels between the anterior and the posterior halves of Clogmia embryos using RNA-sequencing, and found that transcripts of a conserved pair-rule gene, *odd-paired*, are enriched at the anterior pole. In Drosophila, *odd-paired* zygotically regulates the expression of other pair-rule genes during segmentation. We identified three alternative *odd-paired* transcripts in Clogmia (*Cal-opa A, B* and *C*). RNA *in situ* hybridization showed that while *Cal-opa A* and *B* are zygotically expressed like Drosophila *odd-paired*, *Cal-opa* C is expressed only maternally and tightly localized at the anterior pole of freshly laid eggs. Maternal *Cal-opa* C RNAi produced larvae with a mirror-image duplication of abdominal segments ('double-abdomen'). Conversely, injection of *Cal-opa* C mRNA at the posterior pole induced the formation of a second head ('double head'). Thus, *Cal-opa C* is necessary and sufficient for the anterior development in Clogmia. Unlike *bicoid* and *panish, odd-paired* is a highly conserved developmental gene shared across the animal phyla. Our work thus suggests that a conserved developmental gene can rapidly evolve an additional role as a long-range axis determinant through an alternative transcription. Ongoing work focuses on the functional characterization of *Cal-opa C*, and its mechanistic comparison with *bicoid* and *panish.*

554B Classic Meiotic Mapping Meets Illumina Sequencing to Identify New Regulators of *gurken* Translation *Elias Jacobs*, Alexandria Mandriota, John W. Hasper, Scott B. Ferguson. Biology, State University of New York at Fredonia, Fredonia, NY.

Drosophila melanogaster oogenesis relies on the correct localization of *grk* mRNA and it spatially restricted translation . Within the oocyte, Grk specifies the dorsal/ventral patterning of the oocyte which persists through embryogenesis. The *spindle*-class genes are responsible for repairing double stranded breaks (DSB) in meiotic recombination. Mutations in these genes result in inefficient *grk* translation and ventralized eggs. To elucidate the mechanism by which *spn-B* mutations impact *grk* translation, the Schüpbach lab performed an EMS suppressor screen. We are using a combination of traditional meiotic mapping and Illumina sequencing to determine the location of a secondary mutation that is epistatic to *spn-B*^{BU} and will result in the development of wild type eggs. From this screen a selection of six suppressors were outcrossed to generate recombinant lines. From recombinants that still contain the *spn-B*^{BU} mutation, we are able to tell whether or not they suppressed by analyzing their eggshell phenotypes to determine if the epistatic mutation remains or not. A panel of 72 recombinants were sequenced at low coverage on the Illumina HiSeq 2500 platform to identify the precise haplotypes of these recombinant chromosomes and facilitate mapping of the novel suppressors.

555C Semaphorin-1b is required for oocyte re-polarization in *Drosophila melanogaster* *Julia Wittes*, Trudi Schupbach. Department of Molecular Biology, Princeton University, Princeton, NJ.

During *Drosophila* oogenesis, signaling between the germline and somatic follicle cells organizes the embryonic axes. At mid-oogenesis, gurken protein in the oocyte signals through EGFR/torpedo to specify the posterior follicle cells (PFCs). Shortly thereafter, the PFCs signal back to the oocyte, triggering the repolarization of the oocyte microtubule cytoskeleton and the proper localization of the posterior determinant *oskar* and anterior determinant *bicoid*. This signal, known as the posterior signal or polarizing signal, has yet to be identified. We performed an RNAi screen to discover novel posterior signaling genes and identified Semaphorin-1b (Sema-1b). Sema-1b is a member of the Semaphorin family of signaling proteins, which have been implicated in several developmental processes, but which have not yet been shown to function during oogenesis. We report that *Sema-1b* RNAi in the follicle cells causes a failure of oocyte re-polarization but does not compromise overall epithelial integrity or interfere with the specification of the PFCs. We have found that the known Sema-1b receptor Plexin A (PlexA) is expressed in the ovary, and we are currently investigating whether Sema-1b signals via PlexA as part of its role in oocyte re-polarization.

556A Multiple Forkhead (Fkh)-domain proteins repress Bicoid-dependent gene activation in anterior regions of the developing embryo *Timothy R Bishop*, Mary Benac, Stephen Small. Center for Developmental Genetics, New York University, New York, NY.

Bicoid (Bcd) is a homeodomain-containing maternal transcription factor expressed in an anterior gradient along the embryo. Prior to cellularization, Bcd activates a network of segmentation genes that are expressed as broad stripes with anterior and posterior boundaries at specific positions along the anterior-posterior axis. A system of repressors controls the formation of the posterior boundaries of these expression patterns, but little is known about mechanisms that set anterior boundaries or prevent expression at the anterior-most tip of the embryo. In mutational analysis experiments, removing a Slp1 binding site from the eve stripe 2 enhancer caused a derepression of reporter gene in the anterior, but this derepression was not observed in a Slp1 mutant. Also, some Bcd targets, such as otd and hb, are repressed in the anterior pole. This repression is thought to be caused by an activity downstream of the terminal system, but the specific mechanism and transcription factor controlling this repression remains unclear. We identified two previously uncharacterized Fkh-domain proteins with anterior expression patterns— CG9571 and CG11152— that are candidates for repressors in the anterior-most region of the embryo. Misexpressing CG9571 and CG11152 along the ventral surface of the embryo under the control of the Snail promoter showed that CG9571 is sufficient to repress the eve 2 stripe. This result suggests that CG9571 and SlpI may act redundantly to repress the eve 2 stripe in the anterior. We are generating knockouts of these two genes using CRISPR/Cas9 gene editing technology in order to gain a more complete understanding of their role in anterior patterning.

557B Finding transcriptional coregulators of Hox proteins in *Drosophila melanogaster* *Narendra Singh*, Bony De Kumar, Cynthia Staber, Julia Zeitlinger, Kausik Si, Robb Krumlauf. Stowers institute for Medical research, 1000 E, 50th St, Kansas City, MO-64110.

Hox genes regulate the antero-posterior (AP) body axis in all Bilaterians. Diverse studies in many model systems have shown Hox genes play a conserved role in AP patterning. Hox genes encode transcription factors which regulate expression of a large number of downstream target genes during early development. While the conserved role of these proteins is well established little is known about the downstream targets of Hox genes in the genome which are important for their function. A challenge is that Hox proteins have very similar protein sequences and DNA binding domains, which imposes a problem in trying to understand their specificity and in making Hox protein specific antibodies. Recent studies in our group using programmed differentiation of mouse ES cells and epitope-tagged versions of Hox proteins have enabled us to systematically examine targets of mammalian Hox proteins on a genome-wide basis through chromatin immune-precipitation followed by new generation sequencing (ChIP-seq). This work has uncovered novel and overlapping downstream targets of different paralogy groups of Hox proteins. Analyses of binding sequences is also helping to uncover partners and co-factors that may serve to potentiate the activity and regulatory roles of the Hox proteins.

We used specific antibodies against Hox proteins and fly stocks having an epitope-tagged Hox genes. I have mapped the downstream targets of Labial, Antp, Ubx, abd-A and Abd-B in fly genome. We are now in process analyzing the target genes to understand the regulatory mechanisms used by Hox protein. We have also generated specific antibodies against *Drosophila* REST protein and Med19 protein and mapped their downstream target genes. Our results suggest that Mediator, REST and Hox proteins share a large number of common targets in *Drosophila* genome. We have performed various biochemical, cell biology and functional experiments to explore how these proteins along with Hox proteins may participate in regulation of transcription. Our initial results indicate that Mediator and REST complexes work with Hox proteins as transcriptional co-regulators for gene regulation.

558C Elimination of cells with inappropriate positional identity during *Drosophila* wing development *O. Klipa*[1], M. Müller[2], F. Hamaratoglu[1]. 1) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) Biozentrum, University of Basel, Basel,Switzerland.

The compartmentalization and establishment of signalling centres are key processes to organize and coordinate cells during morphogenesis. A maintenance of compartment organization is very important and achieved not only by prevention of cell intermixing but also by removal of mispositioned cells. The mechanisms behind the elimination of such cells are the focus of our study.

In *Drosophila* wing disc the dorsal cells are specified by restricted expression of the selector gene *apterous* (*ap*). We analysed and compared 2 different situations: *ap*$^{-}$ clones in the dorsal compartment and *ap*-expressing clones in the ventral one in a time-course experiment. We found that the early induced (46h AEL) misspecified cells initially were able to proliferate however they were effectively removed afterwards. In this case, wing tissue restored compartment organization before metamorphosis very often. In contrast, late induced clones (66h AEL) were being eliminated less efficiently and a lot of them stayed until the end of larval development. We defined at least 3

mechanisms that are involved in the elimination of the unfit cells: apoptosis, basal extrusion and boundary rearrangement that allows mispositioned cells to be sorted out to the proper side. Interestingly, the contribution of these mechanisms is dependent on clone location within a compartment. For *ap-* clones apoptosis dominates in the dorsal pouch while basal extrusion – in the hinge region. Further, the absence of apoptosis does not prevent the elimination in the pouch, it rather slows down the process using the extrusion as a backup mode. Strong morphological abnormalities in adult wing due to a failure to eliminate mispositioned cells highlight the importance of such mechanisms for tissue plasticity during development.

559A The extracellular protease *AdamTS-B* negatively regulates wing vein patterning through BMP signaling *Mark Schuweiler*[1], Minh Pham[2,3], Afshan Ismat[1]. 1) Department of Biology, University of St. Thomas, St. Paul, MN; 2) Department of Biology, Franklin & Marshall College, Lancaster, PA; 3) present address: Graduate Program in Cellular and Molecular Biology, Baylor College of Medicine, Houston, TX.

Vein patterning in the Drosophila wing provides a powerful tool to study regulation of BMP signaling. Altering levels of BMP effectors can induce major defects in the cross veins and longitudinal veins of wings. The extracellular protease *AdamTS-B* (*CG4096*) encodes an ADAMTS protease expressed in the embryonic trachea and wing imaginal disc precursor cells. Our hypothesis is that *AdamTS-B* controls wing vein formation by interacting with BMP effectors. The absence of *AdamTS-B* displayed an extra PCV vein, and a branched or incomplete distal tip of the longitudinal L5 vein. These defects were similar to over-expression of BMP agonist *crossveinless (cv)*, and down-regulation of the BMP inhibitor *short gastrulation (sog)*. The defects observed in over-expression of *AdamTS-B* were also similar to those of *sog* up-regulation and *cv* knock-down mutations; PCV were not formed completely. These findings suggest that *AdamTS-B* might be a BMP antagonist, modifying BMP agonists or receptors to alter BMP signals. Further study needs to be undertaken to fully unravel interactions between *AdamTS-B* and BMP signaling.

560B Growth regulatory pathway collaborates with axial patterning genes to regulate patterning growth in *Drosophila* eye *N. Gogia*[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3,4]. 1) Department of Biology, University of Dayton, 300 College Park Drive, Dayton, OH; 2) Premedical Program, University of Dayton, 300 College Park Drive, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, 300 College Park Drive, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN, USA.

In any multicellular organism, organogenesis requires axial patterning to determine Antero-Posterior (AP), Dorso-Ventral (DV), Proximo-Distal (PD) axes. Any deviation in these axes during development leads to congenital birth defects. In our model system, *Drosophila melanogaster* (a.k.a fruit fly), Dorso-Ventral (DV) patterning marks first lineage restriction event. We have identified *defective proventriculus* (*dve*-a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1), as a new member of DV patterning gene hierarchy. We have shown that *dve* acts downstream of *pannier* (*pnr*, GATA-1 transcription factor), and upstream of *wingless(wg)* in dorsal gene hierarchy. Loss-of-function of *dve* or *pnr* results in dramatic dorsal eye enlargements, whereas gain-of-function suppresses the eye fate. We have demonstrated that Wg is a downstream target of Hippo growth regulatory pathway (highly conserved) in eye. Furthermore, Wingless (Wg), which acts downstream of *dve*, also exhibits similar eye enlargement and suppression phenotypes and has been shown to play a role in growth. Here, we present that DV patterning genes interact with Hippo signaling to regulate the common downstream target, Wg during growth and patterning of developing *Drosophila* eye. Our data (using Gain-of-function studies) states that activation of Hippo signaling in *dve*, *pnr* expression domain results in change of head specific fate to an eye. We have tested retinal determination fate markers in these backgrounds. This study will address an important question, whether the axial patterning genes (*dve, pnr*) and Hippo pathway regulates patterning and growth independently or in-coordination with each other by regulating Wg signaling in order to form an eye/or any organ. The results from these studies will be presented.

561C Pax6 and Trithorax Group Proteins Together Regulate Cell Fate and Patterning in the Drosophila Eye *Gary Teeters*, Alison Ordway, Justin P. Kumar. Indiana University, Bloomington, IN.

The development of tissues and organs requires that cells be properly differentiated and patterned. When the signals that control patterning are disrupted there are large developmental consequences such as the extra digits seen in polydactyly. My research is focused on the genes that regulate the crystalline-like pattern of the *Drosophila* eye. At the beginning of the third larval instar a wave of morphogenesis sweeps across the epithelium committing progenitor cells to a retinal fate. This wave initiates at the posterior edge of the tissue and progresses anteriorly until it reaches the eye-antennal border. The leading edge of this wave can be visualized by a groove spanning the dorsal and ventral margins call the morphogenetic furrow. For correct retinal patterning only a single furrow can exist. We have discovered that the epigenetic regulators of the Trithorax Group (TrxG) proteins play a role in suppressing the initiation of ectopic furrows. Simultaneous reduction of Twin of Eyeless (Toy), one of two Pax6 genes in flies, and members of the TrxG leads to the initiation of a second furrow from the dorsal anterior margin of the eye field. This region normally gives rise to head epidermis and ocelli originate. This result has led us to primarily

conclude that the combined loss of Pax6 and TrxG are homeotically transforming the head epidermis and ocelli into retinal progenitors. Potential molecular targets for Pax6/TrxG include the Wingless (Wg) signaling pathway since reductions of Wg show very similar phenotypes (Ma and Moses 1995). Other studies have demonstrated that members of the Wg signaling pathway interact with TrxG proteins such as Ash1 and ISWI localizing them to downstream gene targets. These interactions appear important to recruit Trx-G proteins to downstream targets of the Wg pathway. Finally, vertebrate studies show that Pax6 regulates the expression of legless (lgs), a component of the Wg pathway. Together these results lead to a model in which Pax6 and TrxG cooperate with the Wg signaling pathway to limit morphogenetic furrow initiation to a single point at the posterior margin. This regulation is critical for the maintaining the neurocrystalline structure of the *Drosophila* eye.

562A Drosophila Pax6 promotes eye antenna disc development ensuring proper head formation *J. Zhu*, J. Kumar. Department of Biology, Indiana University Bloomington, Bloomington, IN.

Most of the *Drosophila* head structures, including the antenna, ocelli, compound eye, maxillary palps and the surrounding head epidermis are derived from the larval eye antenna disc (EAD). Thus, the EAD serves a good model to study how multiple tissue are differentiated from a single group of progenitor cells during early animal development. While there have been many intense interests in the retina determination gene network controlling eye development, the gene regulatory networks for the rest of the head structures are poorly studied. Here, we show that *Drosophila* Pax6 homologs, *eyeless* (*ey*) and *twin of eyeless* (*toy*) are functionally redundant and are required for the formation of all head structures derived from the EAD by promoting cell survival and tissue proliferation. The loss of Ey and Toy later than 2nd instar larval stage disrupts only the eye development, which is similar to *ey* single mutant phenotype. However, removal of these proteins together in earlier stages disrupts all head structures derived from the EAD. These phenotypes resemble the dosage effect of Pax6 gene in vertebrate eye development. While heterozygous PAX6 mutant results in aniridia in humans and abnormal eye development in mice, complete lack of PAX6 causes embryonic lethality with anophthalmia and severe central nervous system defects. Thanks to well-established genetic tools, *Drosophila* provides an excellent model to understand the role of Pax6 in regulating eye development and neurogenesis. We demonstrate that the control of EAD development by Ey and Toy is mediated through the activation of *teashirt* (*tsh*), *Notch* (*N*) and *eyegone* (*eyg*). In the absence of Ey and Toy, force expression of Tsh, N and Eyg rescue the antenna and head epidermis development to varying degrees, while eye development is not restored. Interestingly, some Tsh rescued fly heads display ectopic wings and thorax epidermis, indicating a segment transformation. These data suggest that the retina genes are not only required for the eye development, but work cooperatively to regulate EAD development during early stages, which ensures proper head formation. Furthermore, previous model suggests that Ey/Toy and N/Eyg control *Drosophila* eye specification and proliferation independently. Here, our new model proposes that, like vertebrate Pax6, *Drosophila* Pax6 genes initiate both processes in eye development. These new findings greatly improve our current understanding of the retina determination gene network in fruitfly and will be very helpful for future research in vertebrate eye development.

563B Identification of systemic injury response genes in *Drosophila melanogaster* *Jennifer Hackney*, Jesus Contreras Rodriguez, Teresa LuPone, Tyler Marsh, Ashley Almajan, Sabahat Hussain, Ty Leek, Jennifer Broatch. School of Mathematical and Natural Sciences, Arizona State University, Glendale, AZ.

Damage to wing imaginal discs early in the third larval instar of *Drosophila melanogaster* induces a systemic response characterized by the activation of specific signaling pathways that facilitate the regeneration of wounded tissue and the coordination of wound healing with organism growth. We induced localized cell death through temperature-dependent expression of *Eiger,* the *Drosophila TNFα* homolog, in wing imaginal discs, then used microarray analysis to identify genes mediating the systemic injury response. A total of 151 genes were differentially expressed in response to wing disc cell death. Further examination of a subset of these genes using chromosomal deficiency lines (Dfs) in combination with genetic wing disc cell ablation identified one of the upregulated genes, *14-3-3ζ* as a potential mediator of tissue regeneration. Larvae containing Dfs that reduced *14-3-3ζ* were subjected to genetic cell ablation. Resulting adults displayed a transformation of the notum into a second set of wings. Together, these results suggest that our analysis may have identified a number of novel systemic injury response genes that mediate tissue regeneration, wound healing and organism growth.

564C Identification of genes that regulate pattern duplication during regeneration *Shilpi Verghese*, Rohan Mylavarapu, Tin Tin Su. Molecular Cellular and Developmental Biology, University of Colorado, Boulder, CO.

Regeneration of tissues after damage requires that the remnant surviving cells proliferate, translocate to the area of tissue damage and re-populate the damaged tissue. The cells need to change and acquire new fate to replenish the lost cells at sites of damage. Rarely, during regeneration events like trans-determination, multiplication and pattern duplication takes place. The mechanism that prevents such errors during regeneration is poorly understood. The cell fate and identity during regeneration is often regulated by epigenetic regulators. A well-controlled regulation of epigenetic regulators is required for regeneration to occur without error. We used wing imaginal discs

of *Drosophila melanogaster* to study the role of epigenetic regulators in regeneration after damage induced by ionizing radiation (X-rays). A hinge specific enhancer was used to mis-express the candidate RNAi transgene in the wing hinge of *Drosophila* larvae and the G-trace system (Evans *et al*., 2009) was used to mark the hinge cells. We found four epigenetic regulators that regulate pattern duplication during regeneration: Set2, Nurf38 and Egg prevent pattern duplication while Enhancer of Polycomb provides an opposing activity. We previously identified that STAT and Wg are required for regeneration. We show here that STAT and Wingless (Wg) are also required for faithful pattern regeneration. These and other data can be fit into a model in which pattern regeneration after irradiation occurs in a set of sequential steps that are regulated by Wg, STAT and epigenetic regulators.

565A Broad and ecdysone are required for the regulation of critical morphogenetic genes in leg discs at the onset of metamorphosis in *Drosophila melanogaster* *Clinton Rice*, Stuart Macdonald, Robert Ward. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

The process of metamorphosis in *Drosophila* is driven by a late larval pulse of the hormone 20-hydroxyecdysone. This hormone is critical for the growth and eversion of the imaginal discs that form the adult integument. Changes in cell shape and cell rearrangements in the imaginal epithelium drive the eversion process and require an ecdysone signaling pathway that includes early-response transcription factors induced by ecdysone, including the zinc finger protein Broad (Br). We previously used a hypomorphic allele of *br* in a genetic modifier screen to identify genes required for leg imaginal disc morphogenesis. Here, we use RNAseq analysis of RNA isolated from leg imaginal discs of prepupae carrying an amorphic allele of *br* (br^5) to identify genes regulated by Br at the onset of morphogenesis. We also conducted RNAseq of late larval and prepupal leg imaginal discs to identify genes that are normally regulated by ecdysone at the onset of metamorphosis. We find that the majority of Br-induced genes are also induced by ecdysone, consistent with the hierarchical model in which Br is induced by ecdysone. However, the majority of Br-repressed genes are not repressed by ecdysone, suggesting an alternate mechanism for Br-based repression. Genes induced by Br include metabolic genes, protease inhibitors, and Notch signaling components, indicating a role for these genes in metamorphosis. The upstream regions of these Br-induced genes are enriched for the various Br binding sites. To determine if these genes play a role in driving elongation and eversion of the leg imaginal disc, we used a *Dll-Gal4* construct to drive RNAi against a subset of the genes induced by Br. We found that malformation of legs resulted from the knockdown of several of these genes, including *CG9416*, the transcriptional activator *grainy head*, and the serine protease *Stubble*. These results indicate that RNAseq analysis of components of morphogenetic pathways is a valid method for identifying genes required in metamorphosis.

566B Mrtf has an early SRF-independent role in adult muscle development *Tracy Dohn*, Richard Cripps. University of New Mexico, Biology Dept. Albuquerque, NM.

SRF and its co-activator Mrtf are known regulators of myogenesis and signaling regulation. Mrtf and SRF are especially prevalent in smooth muscle cell differentiation and are also important factors in cardiac development and repair after injury. SRF and Mrtf are working together late in muscle development to promote flight muscle structure and maturation in Drosophila. However, the role of SRF and Mrtf in early flight muscle development is still not well understood. Therefore, we used RNAi to disrupt SRF and Mrtf in muscle progenitors. SRF RNAi has the same phenotype when it is driven in progenitors or when it is driven in late myotubes in the flight muscles indicating a sole function in late muscle development. However, Mrtf RNAi in muscle progenitors led to lower progenitor cell numbers and subsequently no flight muscle development. Through fluorescent immunostaining of cell death markers in myoblast progenitors in the wing disc we found that there was increased cell death in the wing discs of Mrtf RNAi Drosophila. This indicates a novel role of Mrtf in myoblast survival independent of SRF function which improves our understanding of the mechanism of Mrtf function in muscle development.

567C Abnormal cardiac patterning and development in *akirin* mutant embryos *Madison Hupp*[1], Austin Howard[1,2], Scott J. Nowak[1]. 1) Dept. of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144; 2) Master of Science in Integrative Biology Program, Kennesaw State University, Kennesaw, GA 30144.

Akirin is a highly conserved nuclear transcription co-activator that is essential for proper Twist-regulated gene expression during the embryonic myogenesis program. While Akirin has previously been shown to co-regulate the patterning of the skeletal musculature, recent studies have implicated Akirin as a crucial regulator of the *tinman* locus during specification and patterning of the cardiomyoblasts, the muscle cells that will form the dorsal vessel or heart. *akirin* mutants display a highly disorganized dorsal vessel, marked by missing cardiomyoblasts, and highly aberrant morphology. We are currently employing fixed embryo and live-imaging techniques to image heart formation in *akirin* mutant embryos, as well as dorsal vessel contraction in *akirin* mutants. Our results indicate that *akirin* mutant hearts are patterned abnormally from the onset of cardiac specification, and that the migration of cardiomyoblasts appears to be negatively affected as a result of a loss of Akirin. Given that Akirin is a highly conserved protein among metazoans, it is likely that these results provide a novel mechanism for cardiac specification and patterning that is similarly conserved from insects to mammals.

568A Identification of Akirin-interacting partners during embryonic myogenesis *Kristina Palermino-Rowland*[1,2], Alyssa Griffin[2], Drew Hundertmark[2], Scott J. Nowak[2]. 1) Master of Science in Integrative Biology Program, Kennesaw State University, Kennesaw, GA; 2) Dept. of Molecular and Cellular Biology, 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 musculature. Myoblast population dynamics are tightly controlled by gene expression moderated by this myogenic transcription factor to determine somatic cell fates. Despite the primary importance of myoblast mechanics for building and patterning the musculature, the identities of many molecular players involved in this process remain unknown. Recently we have determined that Akirin, a highly conserved nuclear protein, appears to play a critical role in the regulation of Twist-dependent gene expression during mesodermal specification and muscle development. We hypothesize that Akirin serves as a cofactor to promote interactions between regulatory transcription factors and chromatin remodeling activity to impact gene expression across varying targets. Using a genetic interaction screen in *Drosophila*, we have begun to identify Akirin interacting- proteins that participate in the process of muscle specification, patterning, and development. Our screening method has identified a number of proteins that genetically interact with Akirin during muscle patterning in the embryo. Double heterozygous mutant embryos for *akirin* and one of these potential partners demonstrate a host of deranged or misshapen muscle phenotypes. Thus far we have uncovered a small number of predicted gene products that appear to be involved in general transcription initiation, as well as components of chromatin remodeling complexes. We have extended these studies to map the regions of the Akirin protein that are necessary for such interactions during embryonic muscle development. A candidate region approach using in-vitro translated deletion constructs of Akirin will be taken to investigate the binding capabilities of specific regions across its protein sequence. By characterizing the molecular domain of Akirin function, as well as generating an interactome of its potential partners, we will gain crucial insight into Akirin's mechanism of molecular action during myoblast specification and muscle patterning.

569B Is RAS/MAPK signaling required for muscle founder cell specification? *Sarah Popadowski*[1], Yiyun Zhou[1], Marc Halfon[1,2]. 1) Center of Excellence in Bioinformatics & Life Science, University at Buffalo, SUNY, Buffalo, NY; 2) Roswell Park Cancer Institute, Buffalo NY.

Drosophila muscle development requires undifferentiated myoblasts to be programmed into one of two cell types, founder cells (FCs) and fusion competent myoblasts (FCMs). While both cell types are necessary, it is the founder cells which confer specific identity on the developing muscle fibers. We have observed key differences in the mechanism of founder cell specification between the embryonic somatic and visceral musculature. In both muscle types, activating Ras throughout the mesoderm leads to an increase in FCs at the expense of FCMs, supporting the idea that Ras/MAPK signaling is required for founder cell fate specification. Previous analysis of the somatic FC identity gene *eve* has led to the current model that FC fates are induced by direct activation of FC-specific genes by downstream Ras effectors such as the ETS-domain family member Pointed (Pnt). However, we find that in the visceral mesoderm the role of Ras/MAPK signaling is to relieve repression of the founder cell fate in a subset of visceral myoblasts, a sharp distinction as compared to the activation observed in the somatic musculature. Evidence for this comes from studies of a visceral mesoderm FC-specific enhancer for the *mib2* gene, which drives reporter gene expression throughout both the FC and FCM visceral mesoderm populations when mutated. This expanded *mib2* reporter gene expression is present even in the absence of Ras/MAPK signaling, demonstrating that the requirement for signaling can be circumvented upon de-repression of FC-specific genes. *lameduck* (lmd) mutants also have an expanded visceral mesoderm founder cell complement, which we likewise show occurs even in the absence of Ras/MAPK signaling. We are continuing to define the relationships between *lmd*, Ras/MAPK signaling, and other known or potential members of the muscle development pathway to better define the different mechanisms used for founder cell fate specification in the visceral versus the somatic mesoderm.

570C Identifying Novel Interacting Partners for the UNC-45 Chaperone in *Drosophila melanogaster* *Daniel A. Smith*, Carmen R. Carland, Majid Mekany, Brandon Everly, Sanford I. Bernstein. Biology Dept, SDSU, San Diego, CA.

The UNC-45 protein is a member of the UNC-45/CRO1/She4p (UCS) family that is required for myosin accumulation and thick filament assembly during muscle development in several model organisms. UNC-45 interacts with the chaperone Hsp90 and is 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 have employed a genetic screening approach where *Drosophila* chromosomal deficiency lines are crossed onto an UNC-45 sensitized background. We hypothesize that reduced expression of critical UNC-45 interacting partners coincident with reduced expression of UNC-45 will result in developmental defects that will decrease offspring viability or muscle function. Using this approach, we have identified the deficiency line Df(2R)BSC630, which exhibits impaired flight ability

specifically in the context of reduced expression of UNC-45. Expression of an UNC-45 transgene rescues flight ability in *Df(2R)BSC630; T-33* flies, indicating that the phenotype appears to be specific to the loss of UNC-45. Using overlapping deficiency lines, we have narrowed the genetic window to 12 candidate genes, which we are in the process of investigating using a combination of RNAi knockdown and CRISPR-mediated knockout lines. Additionally, we are developing a transgenic UNC-45:GFP fusion protein that will allow us to isolate UNC-45 complexes that will be interrogated using mass spectrometry. We have shown that the protein is robustly expressed *in vitro* and are currently working to confirm chaperone activity of the UNC-45:GFP fusion protein. We have developed transgenic fly lines that express the UNC-45:GFP construct that will allow us to isolate UNC-45 complexes directly from fly muscle. This biochemical approach for screening UNC-45 interacting partners will complement our genetic approach and further our understanding of UNC-45's role in muscle development. This work has been funded through R01 AR055958 to SIB and F32 AR067654 to DAS.

571A Interaction between Akirin and Mute during embryonic myogenesis *Courtney Willett*, Katharine Majeski, Scott Nowak. Dept. of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

We have recently identified the highly conserved nuclear co-factor Akirin as an essential partner during the process of Twist-mediated gene activation during embryonic myogenesis. *akirin* mutants display multiple defects in muscle patterning, with missing, misattached, and/or duplicated muscles. Live imaging data indicates that the muscles that do form are morphologically thinner and weaker than those observed in wild-type sibling embryos, and that these muscles rapidly deteriorate prior to hatching. These *akirin* mutant phenotypes were reminiscent of *muscles wasted* (*mute*) mutants; the specification, positioning, and patterning of *mute* mutant muscles initially form, but rapidly degenerate as the embryo nears hatching. Despite these phenotypic similarities, a connection between the two had yet to be described. Using a combination of confocal-based live imaging of developing embryos, as well as analyzing whole-mount fixed embryos, we have confirmed a genetic link between these loci. *akirin/mute* double heterozygous mutant embryos display a profound disorganization of the embryonic muscle pattern, with severely degenerated muscles, large numbers of unfused myoblasts, and abnormal patterning and formation of muscle groups in pre-hatching embryos. While a direct interaction between these two gene products is currently under investigation, these data strongly indicate a potential collaboration during the myogenic process.

572B Effect of Nutritional Stress on Fecundity and Maternal Provisioning of Oocytes in *Drosophila melanogaster* *Kamryn N. Gerner-Mauro*[1], Vivian A. Le[1], Lisa M. Goering[2]. 1) Biology, St. Edward's University, Austin, TX; 2) Department of Biological Sciences, St. Edward's University, Austin, TX.

The fitness of an organism is due in part to the available environmental resources. Variability in resources can alter the development and /or physiology of an individual and previous studies in *Drosophila melanogaster* have shown that protein deficiencies can lead to variation in development. In this study, we have raised *D. melanogaster* on diets which vary in the amount of protein. Flies were raised for two generations on normal, low, or high protein food; subsequently, fecundity was measured in an egg-laying assay and maternal provisioning was examined by quantifying the amount of *yolk protein* RNA that was deposited into oocytes. Our predictions were that females raised on low protein food would lay fewer eggs and deposit less *yp* RNA into oocytes than those raised on normal food, while females raised on high protein food would show an increase in fecundity and *yp* RNA deposition. Preliminary results suggest that females raised on low protein food do lay fewer eggs per day. Quantitative PCR assays are still ongoing to determine the effect on *yp* RNA production and deposition in oocytes. Development of both low and high protein diets effected time of development and adult yield. Further studies will examine whether nutritional protein stress affects early development of embryos laid to mothers raised on different diets.

573C *eyeless*/Pax6 Controls Eye Development Through Initiation of the Morphogenetic Furrow *Luke Baker*, Bonnie M. Weasner, Justin Kumar. Biology, Indiana University, Bloomington, IN.

The Pax6 transcription factor Eyeless (Ey) has long been considered to be the master control gene for eye development in *Drosophila*. Loss-of-function mutants identified a century ago were reported to lack compound eyes while more recent studies have shown that forced expression of *ey* in non-retinal tissues is sufficient to induce ectopic eye formation. Additional genes with similar loss and gain of function phenotypes have been identified and shown to comprise the retinal determination (RD) network. This network has been functionally conserved throughout evolution and is thought to regulate the earliest steps in eye/lens development in all seeing animals studied to date. Very few, if any, *ey* mutant flies are actually eyeless – for example, the overwhelmingly number of ey^2 mutant flies have small to medium sized retinas. Publications have studied the variation of eye size in these mutants within the context of environmental influences such as temperature and nutrient availability. Other studies modulated the number of ommatidia in *ey* mutants through genetic interactions with other mutants that affected eye development. None of these studies could adequately explain the variation in eye size. One possible explanation for the variable penetrance of the mutant phenotype is the nature of the *ey* alleles. ey^2 mutants harbor a transposon insertion within an eye-

specific enhancer element. It is possible that these mutants have variable levels of *ey* transcript and this accounts for the variance in eye size. We have deleted the enhancer and observe that only 10% of flies lack eyes while the remaining 90% still have small to medium sized retinas. Our qRT-PCR analysis indicates that the deletion allele (called ey^{LB}) reduces *ey* transcript levels to approximately 2% of wild type levels. Furthermore, the expression of the other fourteen RD genes is relatively normal. These structural and molecular phenotypes of ey^{LB} mutants are at odds with the description of *ey* as being a master regulator of eye development. We have re-examined eye development in *ey* mutants that lack compound eyes and find that the primary cause of the no-eye phenotype is the failure of *decapentaplegic* (*dpp*) to be expressed at the posterior margin which prevents the morphogenetic furrow from initiating pattern formation. We are currently determining if a second Pax6 gene *twin of eyeless* (*toy*) is responsible for *dpp* expression and furrow initiation in *ey* mutant flies that have eyes. Our results challenge the traditional view that Ey controls eye development by acting at the top of the entire eye/lens gene regulatory network. Instead it appears to function in patterning the eye field well after other genes have determined the fate of the retina.

574A How Males and Females Differ: Effector Genes of the Sexual Differentiation Pathway in Drosophila melanogaster *Zach L Farrow*, Artyom Kopp. Evolution and Ecology, UC Davis, Davis, CA.

The sex comb of Drosophila melanogaster is a well studied structure present only in males whose proper development depends on expression of doublesex (dsx), the key transcription factor in somatic sex differentiation. Absence of dsx expression in the T1 leg imaginal disc leads to an intersex phenotype in both males and females. Expression of the male dsx isoform (dsxM) is required to promote sex comb formation while the female isoform (dsxF) is necessary to repress sex comb formation. It is not known how dsxM and dsxF induce this dimorphism. dsx expressing cells will be analyzed in T1 leg imaginal discs of third instar larvae from males, females, and dsx KO flies. Only cells that express or normally express dsx will be included in the analysis. This will be accomplished by combining a nuclear RNA seq method and the Gal4-UAS system. Gal4, under the control of dsx, drives expression of an outer nuclear membrane protein tagged with GFP. This nuclear anchored GFP allows isolation of only cells that normally express dsx. This way only the analogous cells from male, female and intersex flies are analyzed.

575B Trithorax Group proteins interact with Pax6 factor to control proper organ number in the *Drosophila* eye-antennal disc *A.J. Ordway*, G. Teeters, B.P. Weasner, J.P. Kumar. Biology, Indiana University, Bloomington, IN.

Our research focuses on how organ number is controlled during development. For example, mammals most often develop with two eyes and one nose, suggesting a tightly regulated system that is nearly infallible. We are studying how these decisions are controlled in the eye-antennal disc. *Drosophila* larvae have two such discs, each contributing a compound eye, antenna, maxillary palp, one and a half ocelli and parts of the adult head. We aim to understand how interactions between tissue specific transcription factors and epigenetic regulators control organ number. Specifically, we discovered a genetic interaction between members of the Trithorax Group (TrxG) of epigenetic regulators and Twin of Eyeless (Toy), a Pax6 family protein known for its role in eye specification. When these factors are knocked down concurrently, the antennal disc is duplicated leading to animals with four adult antennae.We are focusing on a specific TrxG complex, the Nucleosome Remodeling Factor (NURF). Specifically, we are focusing on how and when Imitation Switch (ISWI) interacts with Toy to limit development to a single antenna per disc. We have characterized the antennal duplication phenotype. Preliminary results suggest that the critical window for suppressing the antennal duplication is during the first instar. We have also discovered that the dorsal head epidermis is absent in double knockdown flies, suggesting that the antennal duplication results from either a fate transformation of the head epidermis, wound healing in response to cell death, or an axis duplication event. We are currently testing the validity of each model. Additionally, we are screening candidate developmental networks that may play a role in this phenotype. Early results show that disrupting the Wingless (wg) pathway phenocopies the ISWI-Toy double knockdown phenotype. Moreover, Wg signaling is known to be involved in both antennal development and wound healing. It is also regulated by TrxG proteins, including NURF. To uncover how Toy and ISWI could be controlling the expression of Wg, we are investigating whether Toy and the TrxG physically interact through the Pax Transactivation Domain Interacting Protein (PTIP). PTIP has been shown to interact with Pax6 in mammals and is known to play a role in *Drosophila* disc development. The PTIP-Toy double knockdown phenocopies the Toy-ISWI double knockdown, suggesting a possible role in the interaction.

In conclusion, my results demonstrate a genetic interaction between highly conserved epigenetic regulators and transcription factors. The loss of these factors together results in serious patterning defects and the duplication of a sense organ.

576C Teashirt and Tiptop control growth, specification, and developmental plasticity of the Drosophila eye-antennal disc *Sneha Palliyil*, Justin Kumar. Indiana University Bloomington.

How the fate of an individual tissue and/or organ is specified remains a fundamental question in developmental biology. Fourteen genes that form the retinal determination (RD) network are responsible for the proper specification

of the Drosophila compound eye and for many aspects of its development. Among them are the zinc finger transcriptional repressors, Teashirt (Tsh) and its paralog, Tiptop (Tio). Our studies suggest that during early development Tsh, alongside Twin of eyeless (Toy), one of the earliest expressing retinal determination network members, functions to maintain cells in a proliferative state. Simultaneous removal of Tsh and Toy early during development leads to pharate lethal adults that are completely headless. Their removal starting after the first larval instar stage leads to other developmental defects such as duplication of the antenna, reduction in eye size, and other miscellaneous head defects. We have evidence showing that these phenotypes result from increased cell death and a reduction in cell proliferation. Interestingly, these effects on eye and head development are not observed when Tio and Toy are reduced together, suggesting that Tsh and Tio may not be functionally redundant during the early eye-antennal disc development. Another central question in organ development is how mutually antagonizing gene regulatory networks control segregation and maintain segregated fates in juxtaposed tissues. The Drosophila eye-antennal disc is an example of such tissue juxtaposition. The eye-antennal imaginal discs give rise to the compound eye, ocelli, antennae, maxillary palps and the head capsule. Our results suggest that maintenance of *tsh/tio* expression in the antennal field after these genes are normally segregated to the eye field leads to continued plasticity of the antennal field. The tissue undergoes a homeotic transformation in which portions of the antenna and head epidermis are converted into an ectopic eye. If Eyeless (Ey) is removed then a segment of the tissue transforms into distal leg segments. Our results also suggest that the mechanism that leads to repression of non-ocular genes, *cut*, *spineless* and *aristaless*, on targeted expression of *tsh/tio* is independent of So, Eya and Ey. Interestingly, we have evidence that the arista to leg phenotype on induction of *tsh/tio* is due to *spineless* repression. These data suggest that Tsh/Tio directly function as regulators of tissue fate.

577A Molecular Fate Mapping of the *Drosophila* Eye-Antennal Imaginal Disc *Bonnie M. Weasner*, Justin P. Kumar. Department of Biology, Indiana University, Bloomington, IN.

The larval eye-antennal imaginal disc is composed of approximately 44,000 cells which give rise to multiple structures in the adult fly including the compound eyes, ocelli, antennae, head epidermis and maxillary palps. A classical developmental study of the eye-antennal disc by Haynie and Bryant in 1986 led to the creation of a generalized fate map detailing regions of the disc responsible for the development of these specific adult structures. Taking advantage of the newly created GAL4 lines from Janelia Farm, we have chosen several independent lines which show distinct expression patterns in the disc corresponding to regions defined in the original fate map. We are first using these lines to drive a UAS-ebony RNAi to determine if the expression pattern in the disc corresponds to a specific structure or region of the adult head. Next by driving a UAS-GFP transgene in these specific regions coupled with fluorescence-activated cell sorting (FACS) to isolate the cells and RNA-seq techniques we are generating a finely detailed molecular fate map of the eye-antennal disc in an effort to better understand the molecules and genetic interactions necessary for the specification and differentiation of those adult structures. Once an RNA-seq profile is generated we will use a combination of RNAi knockdown or CRISPR/CAS9 to generate mutants for candidate genes from each of the fate map regions and assay the effect on the corresponding adult appendage. Taken together these data will provide us with a better understanding of how regional cell fate is specified and maintained in the eye-antennal disc.

578B *Macroglobulin complement-related* (*Mcr*) is required for multiple morphogenetic events during *Drosophila oogenesis* *H. Alhadyian*, L. Ussher, R. Ward. Molecular Biosciences, The University of Kansas, Lawrence, KS.

Macroglobulin complement-related (*Mcr*) encodes a transmembrane protein belonging to the thioester-containing protein (TEP) family and is required for septate junction (SJ) organization and function. SJs are analogous to the vertebrate tight junction in providing an essential occluding function to the epithelium. We initially identified *Mcr* in a screen for genes required for imaginal disc morphogenesis during metamorphosis, and went on to show that animals with homozygous mutations in *Mcr* show embryonic lethality with defects in head involution and dorsal closure; two events that occur prior to the establishment of the SJ. These data suggest a role of *Mcr* in morphogenesis that is independent of its role in the occluding junction. To extend these studies, we are investigating the role of *Mcr* during morphogenetic events that occur during oogenesis. First, we investigated the expression of Mcr in oogenesis, and determined that it is expressed in the germarium, where it is enriched in the germline stem cells. It is also expressed in follicle cells, with the strongest expression in the polar cells. We next used cell-type and stage-specific Gal4 drivers to examine the function of *Mcr* during oogenesis. Reducing *Mcr* in border cells using *slbo-GAL4* to drive *Mcr-RNAi* resulted in defective border cell migration, characterized by incomplete penetrant defects in delamination of border cell clusters, slow migration and cluster disassembly during migration. Reducing *Mcr* in all the follicle cells including follicle stem cells using *GR1-GAL4* resulted in degeneration of middle stages egg chambers, although rounded stage 14 egg chambers were observed that presumably were past stage 8-10 when the RNAi was induced. To examine this further, we reduced *Mcr* in all the follicle cells beginning in stage 8 egg chambers using *c204-*

GAL4, and observed stage 14 egg chambers that were significantly rounder than wild type egg chambers. Together, these observations suggest that *Mcr* plays essential roles in multiple aspects of egg chamber morphogenesis during oogenesis. Further analyses are being conducted to investigate the molecular and cellular mechanisms by which *Mcr* is involved in these morphogenetic processes.

579C Turnover and remodelling of the *Drosophila* heart requires matrix metalloprotease-2 (MMP2). *Chris Hughes*, Roger Jacobs. Biology, McMaster University, Hamilton, Ontario, Canada.

The regulation of extracellular matrix (ECM) turnover by matrix metalloproteinases (MMPs) and their inhibitors is necessary for tissue morphogenesis and homeostasis. Changes in MMP expression are noted in many congenital and age-related vertebrate heart diseases. *Drosophila* possesses two MMPs with partially overlapping substrates: the secreted MMP1 and the transmembrane MMP2. It has previously been demonstrated by our lab that both *Drosophila* MMPs are required during embryonic heart development for normal leading edge membrane motility in migrating cardioblasts, and for regulating lumen size and location. *Drosophila* embryos lacking MMP1 form smaller heart lumens, while those lacking MMP2 fail to form lumens due to the absence of cardioblast junctional domains. Here we report our findings on the function of MMPs during cardiac growth and aging. We employed MMP null mutants (single and double $mmp1^{q112}$ and $mmp2^{w307}$) and targeted RNA interference-mediated knock-down to study the effects of MMP loss of function upon the *Drosophila* larval heart. Examination of MMP2 mutants revealed myofibrillar disorganization and the formation of Collagen-IV (Vkg) aggregates within the heart lumen. Electron microscopy revealed increased ECM deposition on the cardiomyocyte abluminal surface. Confocal and optical coherence tomography (OCT) further revealed a high incidence of bifurcations in MMP2 but not MMP1 mutant dorsal vessels. The bifurcated hearts beat synchronously, and non-bifurcated MMP2 mutant hearts/heart regions did not differ significantly from wild-type in diastolic diameter or stroke volume. Together, these results suggest that, while both MMP1 and MMP2 are necessary for normal heart development and survivorship, MMP2 plays a critical role during lumenogenesis and is required for turnover of the basement membrane throughout growth.

580A Uncovering how Premature Cell Divisions Inhibit Ventral Furrow Formation in *Drosophila* *E*mbryos *Shi Jun Ko*, Adam Martin. Department of Biology, Massachussets Institute of Technology, Cambridge, MA.

An apical actomyosin network promotes apical constriction and epithelial sheet folding in cells with ventral fate during gastrulation in *Drosophila* embryos. In mitosis, cortical actomyosin assembly promotes cell rounding and also forms a cytokinetic ring. How cell cycle state changes the organization of signaling and cytoskeletal proteins in cells with a specific developmental fate is poorly understood in the process of morphogenesis. Embryos mutant for the early zygotic gene *tribbles* (*trbl*) fail to gastrulate due to premature mitotic entry in apically constricting cells, providing a model to investigate molecular reasons for why cell division conflicts with tissue folding. In *trbl* mutant ventral cells, we observed that apical myosin and actin decrease in mitosis, suggesting that mitotic entry disrupts the apical constriction machinery. Currently, we are dissecting the molecular mechanisms for how mitotic entry disassembles apical actomyosin and thus disrupts apical constriction.

581B JAK/STAT signaling is necessary for cell monosis prior to epithelial cell apoptotic extrusion in the follicular epithelium of the Drosophila ovary A. Torres, M. Malartre, F. Agnès, *A. Pret*. Cell Biology Department, Institute of Integrative Biology of the Cell (UMR9198 CNRS/CEA/UPSud) Gif-sur-Yvette/ University of Versailles, France.

Epithelial cell extrusion is crucial for proper development and homeostasis. Studies addressing the modalities of cell remodeling events during epithelial cell extrusion have been conducted in both vertebrate and invertebrate developmental models and in cell culture. These indicate a diversity of extrusion modalities including live and apoptotic cell extrusion, apical and basal extrusion, different types of neighbor exchanges and a role for acto-myosin activity both in the cell to be extruded and in the surrounding epithelial cells. Although real-time imaging has been used in some of these studies, high 3D resolution is lacking to fully comprehend the cell remodeling events within the tissues. In addition, how signaling between cells allows coordination of the events in an epithelium has been addressed in very few cases.

Using high-resolution 3D reconstruction and 4D imaging, we have identified a novel extrusion modality for the elimination of specialized somatic cells from the follicular epithelium in the *Drosophila* ovary. We show here that Polar Cells (PCs) to be eliminated from the follicular epithelium are always fully enveloped by PC neighbors, laterally, apically, in conjunction with highly-reinforced adherens junctions, and basally. The PC to be eliminated thus loses all contact with follicle cells (FCs), germline cells and the basement membrane in a process we have called cell "monosis", for cell "isolation" in Greek. PC monosis always precedes, and is independent of activation of apoptosis. In contrast, monosis requires JAK/STAT signaling non cell-autonomously within FCs. Shortly after monosis is complete, PC apoptotic corpses are formed and extruded laterally within the epithelium to be engulfed by surrounding follicle cells. This study therefore shows the non cell-autonomous impact of an epithelium, via JAK/STAT signaling

activation, on cell morphogenesis events leading to apoptotic extrusion.
Cell and tissue morphogenesis have been shown to involve acto-myosin dynamics in direct interaction with adherens junctions. We have conducted genetic perturbation of Myosin, Cadherin and actin regulators, as well as analysis of the dynamics of these molecules, in PCs and FCs. In addition, we are addressing the mechanical properties of FCs and the role of JAK/STAT signaling in controlling these properties. Results indicate a link between JAK/STAT-signaling control of follicle cell mechanical dynamics and programmed extrusion of PCs.

582C Protein Phosphatase 1 activity promotes a collective rather than single cell mode of migration *Yujun Chen*[1], Damien Ramel[2], George Aranjuez[3], Ashley Burtscher[3], Ketki Sawant[1,3], Maria Lawas[1], Xiaobo Wang[2], Jocelyn A. McDonald[1,3]. 1) Division of Biology, Kansas State University, Manhattan, KS; 2) LBCMCP, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, France; 3) Lerner Research Institute, Cleveland Clinic, Cleveland, OH.
Collective cell migration is prevalent in embryonic development, wound healing, adult tissue renewal and cancer metastasis. While extensive research has been conducted in a variety of models, the underlying mechanisms of collective cell migration are still poorly understood. *Drosophila* border cells undergo a developmentally regulated collective cell migration during oogenesis. Border cells travel as a cohesive cluster of 6-10 cells between large “nurse cells” to reach the oocyte. While roles for several serine-threonine kinases and their target proteins have been established in border cell migration, much less is known about serine-threonine phosphatases. Here we show that Protein Phosphatase 1 (PP1) maintains the collective cohesion and migration of border cells. Inhibition of PP1 activity, either through the endogenous inhibitor NiPP1, or by knockdown of multiple PP1 catalytic subunits, causes border cells to round up and completely dissociate from the cluster during their migration. These individual border cells also have slower overall movement, with protrusions that form randomly between cells. Rac activity is still enriched in the leading border cell, showing that overall guidance signaling and directionality are not affected. However, levels of E- cadherin between cells are strongly reduced. Non-muscle myosin II (myo-II) localization is also altered and F-actin is enriched around individual border cells rather than at the periphery of the entire border cell cluster. Consistent with previous work from the lab on the role of myosin phosphatase, phosphorylated myo-II regulatory light chain is increased in individual border cells. Together, these cellular alterations contribute to the failure of PP1-inhibited border cells to move as a group. Thus, PP1 activity promotes a collective rather than individual mode of cell migration.

583A The role of the ECM protein *fibulin (fbl)* on migration of the caudal visceral mesoderm (CVM) during Drosophila embryogenesis *Olivia Londo*, Anmol Suri, Afshan Ismat. Department of Biology, University of St. Thomas, St. Paul, MN.
Cell migration is a vital process in forming and shaping tissues and organs during embryogenesis. This process requires interactions between migrating cells and the extracellular matrix (ECM), a dense mixture of proteins and macromolecules that surround every cell and tissue. The visceral mesoderm is composed of two layers of cells, the underlying trunk visceral mesoderm (TVM) that goes on to form the circular visceral muscles, and an outer layer of caudal visceral mesoderm (CVM) that goes on to form the longitudinal visceral muscle fibers. The CVM migrates along the underlying TVM, and eventually fuses with certain TVM cells to form the multinucleate longitudinal visceral muscle fibers. The main goal of this study was to begin to elucidate the role of one ECM component expressed in the TVM, *fibulin* (*fbl) (CG31999)*, on the migration of the CVM during embryogenesis. Interestingly, both loss and over-expression of *fbl* in the visceral mesoderm displays gaps in the TVM. Moreover, over-expression of *fbl* in the visceral mesoderm displays uneven migration of the CVM. These findings suggest an important role for the ECM in CVM migration. Further studies need to be conducted to determine the nature of these defects on both the trunk and caudal visceral mesoderm, and the role of the ECM on visceral mesoderm development.

584B Null Mutations in *Imaginal disc growth factor 6* Result in Aberrant Tube Formation During *Drosophila* Oogenesis *Liesl Strand*, Anne Sustar, Celeste Berg. Genome Sciences, University of Washington, Seattle, WA.
From blood vessels to the small intestine to the spinal cord, tubes are an essential part of nearly all multi-cellular organisms. Errors in tube formation cause many of the birth defects that afflict infants, including congenital heart defects and *spina bifida*, a failure to close the neural tube. Our lab uses the fruit fly *Drosophila melanogaster* to study tube formation because of the highly conserved nature of this morphological process between our species. My project focuses specifically on a family of genes called Imaginal Disc Growth Factors (IDGFs), which are linked to tubulogenesis in *Drosophila* and are closely related to a human protein family (CLPs) that have been found to be dysregulated in numerous diseases, including arthritis, and in metastasizing tumor cells. While this homology indicates that IDGFs have a role in cell patterning, the mechanisms by which these genes act remain unclear. Last year, I used CRISPR/Cas9 to investigate the function of one IDGF, the gene *Idgf6*, by deleting it entirely. Analysis of these knock-out mutants suggests that Idgf6 plays an important role in making and shaping tubes and that removal of

Idgf6 results in branched structures instead of discrete tubes. This branching phenotype likely limits the flow of oxygen to the developing embryo, resulting in decreased survival of offspring. My current research explores this genetic pathway further by using antibody staining to determine the mechanism of tube dysfunction in *Idgf6* mutants, and to identify other pathways that interact with Idgf6 during tubulogenesis.

585C The extracellular protease *AdamTS-B* plays an important role in tracheal migration during Drosophila embryogenesis *Abigail Thuringer*, Samantha Leibold, Afshan Ismat. Department of Biology, University of St. Thomas, St. Paul, MN.

Proper organ formation requires certain cell types to migrate from one place in the embryo to another. Cells migrate through a dense extracellular matrix (ECM) filled with proteins and macromolecules. Many different types of proteins are used to assist cells in their migration through the ECM, including extracellular proteases that cleave, or restructure, parts of the dense ECM to allow cells to move through it. One family of extracellular proteases is the ADAMTS family, known to play important roles in cell migration. Drosophila *AdamTS-B (CG4096)* is expressed in the embryonic trachea from early to late stages of tracheal development. The trachea is a highly branched network of tubular airways that go through an elaborate migration throughout embryogenesis. Embryos completely missing *AdamTS-B* displayed luminal cysts as well as discontinuous lumens with gaps throughout tracheal branches. Conversely, over-expressing *AdamTS-B* throughout the trachea showed several extra branches and extra long branches. Interestingly, over-expressing *AdamTS-B* throughout the trachea also displayed luminal cysts, similar to those seen in the loss of *AdamTS-B*. Taking these data together demonstrate an important role for this extracellular protease *AdamTS-B* in proper tracheal development.

586A Same cells, different tubes: Dorsal appendage formation in Drosophila and Scaptodrosophila *Rachel Dam*, Kenley O'Hanlon, Sophie Archembault, Celeste Berg. University of Washington, Seattle, WA.

How do two species employ a conserved developmental pathway to create different structures from epithelial sheets? Tubulogenesis gives rise to the gut, heart, and neural tube in many species during development. These organs form through a common wrapping mechanism to create a tube parallel to the original epithelial sheet. During oogenesis in *Drosophila melanogaster*, two patches of follicle cells overlying the developing oocyte employ the same wrapping mechanism to create specialized eggshell structures called dorsal appendages (DAs). Surprisingly, however, in a related species *Scaptodrosophila lebanonensis*, cells crawl anteriorly instead of wrapping, and they drag more posterior cells with them to create a variable number of DAs. To investigate the molecular mechanisms that facilitate these distinct tube-forming processes, we began by examining how the epidermal growth factor (EGF) pathway, known to play a role in patterning tube-forming cells in *Drosophila*, contributes to DA formation in *Scaptodrosophila.* Using protein BLAST, we identified *Scaptodrosophila* orthologs of *Drosophila* genes encoding EGF pathway components and cloned the genes following PCR. We compared mRNA expression levels and patterns by *in situ* hybridization and found that *Scaptodrosophila* creates its variable number of DAs from a single primordium. Analyses of transcripts from *broad*, *argos*, *pointed*, and *thickvein*, as well as immunohistochemistry against Capicua and phosphorylated Mad showed that the earliest steps in follicle-cell patterning differ in these species. Oocyte expression of *gurken*, which encodes the EGF ligand, was marginally more expanded in *Scaptodrosophila*, but the slightly thicker domain of expression cannot explain the overall differences in primordia patterning between these species. We speculate that differences in the Gurken amino acid sequence elicit distinct responses in the follicle cells of these two species. To test this hypothesis, we are expressing the *Scaptodrosophila gurken* ortholog in *Drosophila melanogaster* and examining dorsal-appendage formation and primordia patterning. These studies will yield insight into how changes in the EGF pathway have facilitated morphological evolution in two Drosophilid species.

587B Possible Functions of Obscurin (Unc-89) in Non-muscle Tissue *Anja Katzemich*, Frieder Schöck. Department of Biology, McGill University, Montreal, QC, Canada.

Obscurin is a large, multi-domain protein, originally identified in vertebrate striated muscles and part of the titin family. Members of this family are made up of tandem immunoglobulin (Ig) and fibronectin-like (Fn3) domains and can have one or two kinase domains near the C-terminus. Other signaling domains, such as Rho guanine nucleotide exchange factors (Rho-GEFs), can also be present. In the muscle sarcomere, obscurin predominantly localizes to the M-line and plays essential roles in myofibrillogenesis, cytoskeletal organization, and the proper symmetry and alignment of myosin thick filaments. Obscurin has a similar modular structure in invertebrates and vertebrates, but the number of modules and the position of signaling domains vary. The largest isoform of *Drosophila* obscurin comprises single SH3 and Rho-GEF signaling domains at the N-terminus and two kinase domains at the C-terminus, as well as 21 Ig-domains. With the generation of specific antibodies directed against epitopes in the N- and C-terminus as well as the middle of the protein, we have undertaken a systematic approach to characterize the expression profile of

obscurin in muscle and non-muscle tissue of *Drosophila*. While big isoforms of ~475 kDa are predominantly expressed in muscle tissue, smaller isoforms ranging from 250 kDa to 48 kDa, are mainly expressed in non-muscle tissue, such as the salivary glands, imaginal discs, as well as in the early and late stages of the embryo. Different isoforms are present in different subcellular locations, including epithelial membranes and nuclei, suggesting additional roles for obscurin in nuclear architecture and epithelial organization. In wing and leg discs of third instar larvae as well as early pupae, obscurin is expressed in a similar localization pattern as non-muscle myosin II. While non-muscle myosin II drives the contraction of cells during disc folding and eversion, obscurin may be needed to organize the bipolar filaments of non-muscle myosin in epithelial cells. Co-immunoprecipitation demonstrated that both proteins are associated. Knocking down obscurin in the wing and leg disc using RNAi lead to malformed legs and small wings. Available hypomorphic alleles of obscurin show similar phenotypes. Currently, mutagenesis approaches including the CRISPR/Cas9 system are being undertaken, in order to further analyze the function of obscurin in different tissues and cellular processes.

588C Characterization of eggshell diversity among *Drosophilidae* species: signaling, patterning, and morphogenesis *Alessio Russomanno*[1], Nir Yakoby[1,2]. 1) Biology, Rutgers, Camden, NJ; 2) Center for Computational and Integrative Biology,Rutgers, Camden, NJ.

The *Drosophilidae* eggshell is structurally diverse among species and thus has emerged as an exciting system to study the evolution of morphologies. Attention has been given to study of the mechanisms of dorsal appendage formation. The development of other structures, including the lumen-like structure, the dorsal ridge, has been recently studied in our lab. In addition, extending our interest to *Scaptomyza,* a genus of *Drosophilidae* closely related to Hawaiian *Drosophila*, revealed a previously uninvestigated structure along the dorsal side of the eggshell known as the respiratory stripe. We present a comprehensive eggshell analysis of *S. anomala* and *S. elmoi* (flies with a respiratory stipe), *D. nebulosa* (a fly with a dorsal ridge), and *D. melanogaster*. Eggshells are derived from a monolayer of epithelial cells, the follicle cells, engulfing the developing oocyte. In *Drosophila*, follicle cell development relies on numerous cell signaling pathways, including the epidermal growth factor receptor (EGFR) and bone morphogenetic protein (BMP) signaling pathways in which the latter controls anterior structures. Interestingly, we found reduced BMP signaling in flies with a small operculum. Furthermore, we show that the dynamics of EGFR signaling are consistent with the diversity of eggshell structures. We also found dramatic changes in follicle cell patterning, including the operculum marker Fasiclin-III and the dorsal appendage marker Broad. Finally, we demonstrate that, while the disruption of EGFR signaling eliminates the respiratory stripe in *Scaptomyza* species, lateral features still remain.

589A Cytonemes Mediate Contact-Dependent FGF Dispersion And Gradient Formation Lijuan Du, *Sougata Roy*. University of Maryland, College Park, College Park, MD.

The mechanism by which Fibroblast Growth Factor (FGF, Branchless/Bnl), one of the key signaling proteins in metazoan development, disperse in the cells of the developing branches of Drosophila trachea is not known. We created genome edited Bnl:GFP (FGF) and its receptor Brtealess-Cherry (Btl:Cherry) fusions and showed the cytonemes from trachea receive Bnl in a contact dependent manner. Cytonemes from both recipeint and producing cells contact one another and exchange the signals at the point of contact. Bnl binds to its receptors and move along the cytonemes. Cytoneme mediated transport creates both extra- and intra- cellular Bnl gradient and graded distribution of Bnl creates branching pattern. Understanding the control mechanisms that cells employ to communicate and interpret signaling proteins such as FGF Will have profound implications in our understanding of both normal development and diseases.

590B Analysis of Imaginal Disc Growth Factors (IDGF) mutants *Anne E. Sustar*, Liesl G. Strand, Sandra G. Zimmerman, Celeste A. Berg. Dept of Genome Sciences, Univ of Washington, Seattle, WA.

Drosophila Imaginal Disc Growth Factors (IDGFs) induce dramatic growth, polarity, and cell migration in cell culture (Kawamura et al., 1999). The human homologs, chitinase-like proteins (CLPs), are necessary for wound healing and immune response, and their upregulation is associated with tumor metastasis and chronic inflammatory diseases. Despite their potential medical importance, however, little is known about this family of genes compared to other growth factors, and the receptor and other interacting proteins are unknown.

Flies have six *IDGF* genes, and overall research progress has been limited by the lack of mutants. Therefore we created null deletions using CRISPR-Cas9. We observe that flies that are mutant for any single *IDGF* are viable and fertile. With the exception of low-penetrance eggshell defects in the *IDGF6* mutant, the mutants have no visible phenotypes. We reasoned that the six *IDGF*s could be partially redundant. Indeed, qPCR revealed that mutating one *IDGF* induces others to upregulate their expression.

To uncover the common function of IDGFs, we recombined our mutants to make sextuple-mutant flies. In the sextuple-mutant flies, we observe low-penetrance lethality throughout all stages of larval and pupal development. About 20% of sextuple-mutant fly eggs exhibit defects in dorsal-appendage morphology. In older adult flies we observe occasional abdomen melanization that is consistent with infections. A more penetrant phenotype is etched tergites on adult abdomens. Nevertheless we were surprised to find that flies that lack all IDGFs are largely viable and fertile.

Why would a gene family be maintained throughout evolution if its products are not required? The phenotypes that we observe are consistent with IDGFs' putative roles in tissue remodeling, cell adhesion, and immune response. Although these phenotypes in the laboratory are subtle, we propose that IDGFs may be more necessary in the wild, where flies are challenged with non-optimal conditions such as poor nutrition and pathogens. We will test these possibilities.

Additionally, we cannot exclude the possibility that the six IDGF genes are redundant with other genes that have yet to be identified

591C Downstream targets of forkhead domain transcription factors mediating cardiogenesis *Andrew J. Kump*[1,2], Manoj Panta[1,2], Srivathsan V. Raghavan[1,2], Ye Chen[3], Xujing Wang[3], Neal Jeffries[3], Shaad M. Ahmad[1,2]. 1) Department of Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN; 3) National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

While mutations in four genes encoding Forkhead (Fkh/Fox) domain transcription factors (TFs) have been linked to human congenital heart defects, little is known about the molecular mechanisms or the downstream target genes by which these Fkh TF-mediated cardiogenic functions are brought about. Our prior work identified the cardiogenic roles of two *Drosophila* Fkh genes, *jumeau* (*jumu*) and *Checkpoint suppressor homologue* (*CHES-1-like*), mutations in which result in disruptive changes in cardiac cell types and numbers, defects in cardiac progenitor cell specification and division, and errors in cardiac cell position. Furthermore, Fkh TF binding sites are significantly enriched in the enhancers of cardiac genes. Collectively, these results indicate that these two Fkh TFs integrate diverse cardiogenic processes by regulating large numbers of downstream target genes, thus raising the question of what these target genes are and what their individual functions might be during heart development. Utilizing RNA-sequencing to compare genome-wide transcriptional expression profiles of flow cytometry-purified mesodermal cells from wild-type, *jumu* loss-of-function, *CHES-1-like* loss-of-function, and *jumu* and *CHES-1-like* dual loss-of-function embryos, we identified 2,130 putative Fkh targets, i.e. genes exhibiting significant differential expression in *jumu* and/or *CHES-1-like* mutants compared to wild-type. To assess their potential cardiogenic roles, we have begun phenotypic analysis of RNAi knockdowns and loss-of-function mutations of a prioritized subset of these genes. Here we present some examples of Fkh target genes involved in cardiac mesoderm specification, cardiac progenitor cell division, and correct positioning of cardiac cells. In addition, we show that cardiac cell positioning errors in Fkh mutants are not solely due to steric constraints imposed by contralateral hemisegments possessing unequal number of cardiac cells as a consequence of defective cardiac progenitor cell divisions, indicating a role for additional categories of Fkh targets.

592A Uncoupling apical constriction from tissue invagination *Se-Yeon Chung*, Sangjoon Kim, Deborah Andrew. Dept Cell Biol, Johns Hopkins Univ, Baltimore, MD.

Apical constriction is a widely utilized cell shape change linked to the folding, bending and invagination of polarized epithelia. It remains unclear how apical constriction is regulated spatiotemporally during tissue invagination and how much this cellular process contributes to tube formation in different developmental contexts. Using the *Drosophila* salivary gland (SG) invagination as a model, we show that regulation of *folded gastrulation* (*fog*) expression by the FoxA transcription factor Fork head in the SG is required for apicomedial accumulation of Rho kinase and non-muscle myosin II, which coordinate apical constriction. We demonstrate that neither the loss of spatially coordinated apical constriction nor its complete blockage prevent SG internalization and tube formation, although such manipulations do affect the geometry of the invagination pit. We further demonstrate that fully elongated and properly polarized SGs can form outside the embryo, suggesting that tube formation and elongation are intrinsic properties of the developing SG.

593B The *speck* gene, first identified in 1910, is a mutation in a transcript of the *Dopamine acetyltransferase* (*Dat*) gene. *Eric Spana*. Department of Biology, Duke University, Durham, NC.

The pigmentation mutation *speck* (*sp*) is a commonly used recombination marker characterized by a darkly pigmented region at 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 115 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 acetyl-transferase (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.

594C Terminal Axon Arborization In The Neuromuscular Junction Is Critically Dependent On Neuronal Expression Of Miles-To-Go (Mtgo), A Drosophila FNDC3-Like Protein *J L. Marsh*[1], T. Lukacsovich[1], A. Syed[1], M. Pomeroy[1], S. Worthge[1], S. Wei[1], J. Purcell[1], C. Park[1], K. Waymire[1], G. Decker[2], T. B. PHam[1], J. Gui[1], A. Paul[1], E. Padilla[1], J. Bardwell[1], R. Zebarjedi[1], B. Zhang[2], L. Bardwell[1], G. MacGregor[1]. 1) Dept Dev & Cell Biol, Univ California, Irvine, Irvine, CA; 2) Division of Biological Sciences, University of Missouri, Columbia, MO 65211-7400.

Despite progress that has identified many signaling molecules and transcription factors that specify cellular identity, little is known about the factors that actually execute the morphogenetic programs leading to organogenesis. The neuromuscular junction is a highly dynamic structure that grows or retracts as the synapse responds to input. We describe studies indicating that a Drosophila FNDC3-like protein (named miles-to-go, mtgo) partners with a chaperonin and is required for terminal arborization of type I neurons at the NMJ. Unbranched type II neurons that drive larval crawling are unaffected. *mtgo* mutants exhibit late pupal lethality that is rescued by expression of *mtgo* in neurons but not muscles. *mtgo* mutants exhibit the most severe disruption of the NMJ that we have been able to find in the literature to date. Mutant larvae exhibit behavioral deficits including defective chemotaxis, loss of sweeping behavior during food search and disruption of the ability to roll over although no defect in crawling was observed consistent with the fact that type II neurons appear unaffected. Electrophysiology studies reveal a reduced EJP amplitude and mini frequency but no reduction in the mini amplitude consistent with a presynaptic deficit. Binding studies confirm that MTGO binds weakly to dCCTg a chaperonin subunit that was identified in a functional screen for tracheal branching mutants and in a biochemical screen for binding partners. Consistent with this dCCTg mutants (*vine*) exhibit a synthetic phenotype when doubly heterozygotes with *mtgo* (*mtgo*/+;*vin*/+) and *vin* heterozygotes exhibit NMJ defects similar to those seen in *mtgo* mutants. We propose that mtgo and vin comprise part of a molecular cytoskeletal organizing complex that promotes branching morphogenesis at the NMJ.

595A Self-organized Notch dynamics generate stereotyped sensory organ patterns *Francois Schweisguth*[1], Lydie Couturier[1], Herve Rouault[1], Khalil Mazouni[1], Francis Corson[2]. 1) Institut Pasteur, CNRS UMR3738, Paris, France; 2) LPS, Ecole Normale Superieure, CNRS, Paris, France.

The emergence of spatial patterns in developing multicellular organisms relies on positional cues and cell-cell communication. *Drosophila* sensory organs have informed a paradigm where these operate in two distinct steps: prepattern factors drive localized proneural activity, then Notch-mediated lateral inhibition singles out neural precursors. Whether self-organization might also contribute to proneural patterns remains unexplored. Using a combination of experiments and modeling, we show here that Notch signaling dynamically organizes a sequence of proneural stripes that resolve into regular rows of sensory bristles on the fly thorax. Patterning is initiated by a broad gradient of Delta ligand expression, then progresses through inhibitory signaling between and within the stripes. Our study reveals that Notch signaling can support self-organized patterning on a tissue-wide scale, and provides a simple example where a broad prepattern is transduced by cell-cell interactions to produce an elaborate arrangement of fates.

596B Transcriptional bursting in *Drosophila* development: stochastic dynamics of *eve* stripe 2 expression *David Holloway*[1], Alexander Spirov[2]. 1) Dept Mathematics, British Col Inst Tech, Burnaby, BC, Canada; 2) Sechenov Institute of Evolutionary Physiology, St. Petersburg, Russia.

Body segmentation in *Drosophila* is first seen in the 7-stripe expression patterns of the pair-rule genes. Recent live imaging of the *even-skipped* (*eve*) pair-rule gene (using a reporter for stripe 2 expression) shows that its expression is noisy, with ‘bursts’ in the number of RNA transcripts being made over time. We developed a stochastic model to analyze the noisy experimental time series and test hypotheses for how *eve* transcription is regulated. These include whether *eve* is transcribed with a simple ON-OFF mechanism with a single ON rate, or by a more complex mechanism with multiple ON rates. We find that both mechanisms can produce long (multi-minute) RNA bursts, but that the short-time (minute-to-minute) statistics of the data is indicative of *eve* being transcribed with at least two distinct ON rates. This is consistent with data on the joint activation of *eve* by the Bicoid and Hunchback proteins. We also predict distinct statistical signatures for cases in which *eve* is repressed (e.g. along the edges of the stripe) vs. cases in which activation is reduced (e.g. by mutagenesis of transcription factor binding sites). Fundamental

developmental processes such as gene transcription are intrinsically noisy; our approach presents a new way to quantify and analyze data from such phenomena, for understanding regulatory mechanisms and how they propagate noise and impact developmental robustness.

597C Dynamics of BMP gradient formation and interpretation *A. Beseli*, G. Reeves. Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

The BMP signaling network regulates a wide variety of cellular responses in most animals from flies to humans. In flies, one of the functions of the BMP signaling network is to determine dorsal-ventral patterning in the early embryo. BMP signaling works like a morphogen, where BMP target genes are expressed in at least three nested domains, implying three concentration thresholds (ct1-3). Starting with early nuclear cycle (nc) 14, low levels of BMP signaling on the dorsal-most 25% of the embryo pass the first concentration threshold (ct1) to activate low-threshold genes (such as *pnr*), which leads to the differentiation of this region into dorsal epidermis. In mid and late nc 14, BMP signaling intensifies, but also contracts to first pass ct2 and then ct3 on the dorsal-most 12% and 5% of the embryo, respectively, causing the activation of mid- and high-threshold genes. However, low-threshold genes remain active in 25% of the embryo, even though the signal is lost outside the dorsal-most 12% and 5% of the embryo. To explain how these genes remain active after BMP signaling is lost, a hypothesis was generated that denies the BMP signal loss in the later nc 14 and claims that a low, undetectable level of BMP signal above ct1 remains all through nc 14. As an alternative hypothesis, there might be memory transducers activated by BMP signaling above ct1 in the early nc 14 that keep the BMP target genes active through nc 14 even after BMP signal would be lost. To test this hypothesis, we are first working on determining whether the enhancers of the target genes remain transcriptionally active in the later stages of nc 14. Using live imaging, we show that the enhancer of one of the target genes, *pnr*, was detected to be active throughout nc14. The enhancers of more of the target genes are currently being tested. Furthermore, the dynamics of BMP signaling in nc 14 are monitored in live embryos to determine whether signaling above ct1 is lost outside the dorsal-most 12% and 5% of the embryo. With the help of these results the mechanisms overlying the patterning and development in animals can be better understood.

598A Prospero promotes scolopale cell differentiation by repressing cap cell differentiation genes in the chordotonal organ *Adel Dunayevskyy*[1,2], Adi Salzberg[1,2]. 1) Department of Genetics, Rappaport Faculty of Medicine and Research Institute; 2) Technion-Israel Institute of Technology, Haifa, Israel.

Prospero (Pros) is a homeodomain transcription factor known for its important role in the development of the central nervous system, where its nuclear activity specifies the ganglion mother cell fate. In the embryonic peripheral nervous system (PNS), however, Pros has been thought to affect only axonal pathfinding, but not cell fate determination. Here we show for the first time that *pros* plays a critical role in cell fate diversification within the chordotonal organ (ChO) lineage.

Each ChO in the larval pentascolopidial organ is constructed as an array of five clonally related cells with distinct identities (cap-attachment, cap, scolopale, neuron, ligament). These cells originate from a single ChO precursor through four rounds of asymmetric cell divisions. Using a battery of cell type-specific markers we demonstrate that Pros is required for promoting the scolopale-specific and for repressing the cap-specific differentiation programs.

One of the target genes affected by Pros activity is *delilah* (*dei*). The *dei* gene is normally expressed in the attachment cells as well as the cap and ligament cells of the ChO, but is excluded from the scolopale cell and the neuron. In *pros* mutant embryos *dei*'s expression expands into the *pros*-negative scolopale cells. In contrast, cap cells that are forced to express *pros* adopt a scolopale-like phenotype; they lose *dei*'s expression and manifest scolopale-specific structures (e.g. scolopale rods). These observations suggest that, normally, Pros represses *dei* in the scolopale cell. The identification of a regulatory module within the *dei* locus through which Pros regulates *dei*'s expression corroborates this idea. We assume that this repression is critical for scolopale cell differentiation and for preventing the scolopale cell from adopting cap cell-specific features.

599B Expression of *d4* and *tth* Genes during *Drosophila* Development *I. Mertsalov*, D. Kulikova, R. Cherezov, O. Simonova. Lab of Regulation of morphogenesis, Koltzov Institute of developmental biology RAS, Moscow, Russian Federation.

Two *Drosophila* genes *d4* (*CG2682*) and *tth* (*CG12175*) belong to an evolutionarily conserved *d4* (*Dpf*) gene family, differentially expressed in the central nervous system during the development. The proteins encoding by members of *d4* (*Dpf*) gene family contain two conserved domains: N-terminal 2/3 domain and C-terminal double paired PHD fingers (D4 domain or Dpf), however *Drosophila tth* (*CG12175*) contains 2/3 domain only. In mammals the D4/Dpf proteins are associated with neuronal SWI/SNF chromatin remodeling complex BAF and their C-terminal domain is responsible for binding to nucleosome's methylated and acetylated lysines of histones 3 and 4. It was observed that in animal evolution the increase of copies of the D4/Dpf homologs from single one in *hydra* to three in mammals as well as the emergence of alternative promoters and splice variants occurs. Perhaps this reflects the complexity of nervous system organization formed during the evolution. The *d4* gene has two alternative promoters. The transcripts

synthesized from distant promoter have 6 exons spanning 37 kb whereas the transcripts synthesized from close promoter have 3 main coding exons spanning 1,98 kb. Using 5' RACE we have identified mRNAs analogous to CG2682- A,C,D sequences in NCBI database. These splice variants synthesized from close promoter encode two-domain proteins with small differences in length. We have found that both promoters are active at all stages of *Drosophila* development however the mRNAs transcribed from distant promoter are expressed at significantly lower level. Using affinity mRNA purification with ssDNA probe specific to *Exon 2* of the distant promoter transcript followed by 5'RACE we have identified three novel mRNAs and previously described isoform CG2682-B. The novel transcripts encode full-length protein, truncated protein without 2/3 N-terminal domain and truncated novel protein that contains additional predicted N-terminal peptide respectively. Specific activity of alternative promoters of *d4* gene in different types of neurons has been proposed. The *tth* gene has single transcript *CG12175*. Using specific antibodies raised against TTH and D4 we have studied the expression of *d4* and *tth* genes at all stages of *Drosophila* development including early embryonic development and precellular blastoderm stage. We demonstrated that both proteins are localized in interphase nuclei but they are not detected during metaphase. To determine *tth* loss of function effect on *Drosophila* neural development the knockout of *tth* gene was performed according to protocol of ends-out gene replacement (K.Golic, PNAS USA, 2003). We have found that *tth* disruption led to 50% embryo mortality due to malformation of optical lobes in embryonic brain.

600C *In vivo* expansion of functionally integrating GABAergic interneurons by targeted increase of neural progenitors Rachel E. Shaw[1], Benjamin Kottler[2,3], Zoe N. Ludlow[2,3], Dongwook Kim[2,3], Sara Morais da Silva[4], Edgar Buhl[5,6], James J.L. Hodge[5], Frank Hirth[2,3], *Rita Sousa-Nunes*[1]. 1) MRC Centre for Developmental Neurobiology, King's College London, UK; 2) MRC Centre for Neurodegeneration Research, King's College London, UK; 3) Department of Basic and Clinical Neuroscience, King's College London, UK; 4) Department of Physiology, Development and Neuroscience, University of Cambridge, UK; 5) School of Physiology and Pharmacology, University of Bristol, UK; 6) University of Exeter Medical School, UK.

Amplification of defined neuronal populations is a key goal for regenerative medicine. Current applications rely on low-yield *in vitro* differentiation protocols and uncertain circuit integration. This proof-of-principle study reports *in vivo* amplification of lineage-specific neurons that incorporate into neural circuitry without cancer induction nor detriment to animal behavior. Central brain-specific lineages in *Drosophila* were targeted to attenuate expression levels of the cell fate determinant Prospero (Pros) within a range that expands not only progenitors but also neuronal progeny. The resulting supernumerary neural stem cells (NSCs) undergo functional transitions similar to wild-type NSCs, progress through the temporal patterning cascade and generate progeny characterized by molecular signatures and circuit integration concordant with source lineages. In particular, fully differentiated supernumerary gamma-amino butyric acid (GABA)-ergic interneurons form neuronal connections in the central complex of the adult brain. *In vivo* calcium imaging and open-field behavioral analysis establish their functional integration into neural circuitry required for higher motor control.

601A The role of CDK8-CycC in regulation of *Drosophila* wing vein patterning *Xiao Li*, Qun Wang, Xinsheng Gao, Jun-Yuan Ji. Department of Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX.

Dysregulation of CDK8 (Cyclin-Dependent Kinase 8) and its regulatory partner CycC (Cyclin C) has been identified in a variety of human cancers. For example, amplification or overexpression of CDK8 gene is observed frequently in melanoma and colorectal cancer patients. Overexpression of CDK8 alone can promote the growth of these two types of cancer. Importantly, the growth of melanoma or colorectal cancer cells with gained CDK8 activity is potently blocked when CDK8 is inhibited or depleted. These observations suggest that CDK8 is likely an oncoprotein in melanoma and colorectal cancers, making it a promising drug target. However, to successfully develop pharmaceutical drugs that target CDK8, it is essential to understand the regulatory network of CDK8-CycC in both normal development and tumorigenesis. To identify upstream regulators or downstream effectors of CDK8-CycC, we performed a dominant modifier genetic screen based on the vein patterning defects caused by the overexpression or depletion of CDK8-CycC. Further mapping of a few enhancers and suppressors allowed us to identify the genetic interactions between CDK8-CycC and EGFR as well as Dpp (TGFβ in *Drosophila*) signaling pathways. Both EGFR and TGFβ signaling pathways are essential for normal development in metazoans, and are frequently mutated in human cancers. The genetic interactions between CDK8-CycC and these two signaling pathways in patterning *Drosophila* wing veins provide a basis for the further investigation of the molecular mechanisms underpinning these interactions *in vivo*, and the examination of whether these mechanisms are conserved in mammals, particularly in human cancer cells.

602B Examining the role of promoter-proximal BEAF in gene expression. *Yuankai Dong*, Satya Avva, Craig Hart. Dept of Biological Sciences, LSU, Baton Rouge, LA.

BEAF (Boundary Element-Associated Factor) was originally identified as a chromatin domain insulator binding

protein, suggesting that it might play a role in linking gene regulation to chromatin organization and dynamics. Genome-wide mapping found that BEAF is usually found near transcription start sites, often of housekeeping genes, suggesting that it might play a role in promoter function. This would be a nontraditional role for an insulator binding protein. To gain insight into molecular mechanisms of BEAF function, we identified proteins that interact with BEAF using yeast 2-hybrid assays. The proteins we identified include some transcription factors. The transcription factor interactions were confirmed in pull-down experiments using bacterially expressed proteins, and in a genetic assay based on a rough eye phenotype in transgenic flies. We are currently using luciferase assays in S2 cells to test the significance of these interactions for gene expression.

603C Cell-fate specification in the accessory glands of *Drosophila melanogaster* *Giovanni Hanna*, Olga Barmina, Artyom Kopp. College of Biological Sciences, UC Davis, Davis, CA.

Although much is known regarding cell-fate specification and its role in development, less is known regarding the evolution of cell specialization across species and its effect on organ complexity and function. The accessory glands in male Drosophila serve as a great model to answer questions related to the rise and evolution of cellular complexity across Drosophilids, and can help us understand the processes that regulate differentiation of cell types from progenitor cells. Two cell types in the accessory glands, the primary and secondary cells, are different in size, abundance, and function across Drosophila. What transcription factors maintain the primary and secondary cell fates seen in adult flies? How many of these are shared across the two cell types? And what factors contribute to specialization of the cells from progenitor cells? RNA-seq data allowed us to identify transcription factors expressed in this paired organ, and subsequent studies are helping us generate regulatory networks in each of these cell types.

604A The gene *tfiia-s-2* encodes a testis-specific TFIIA subunit homolog in *Drosophila melanogaster* *Joseph S Mohammadi*, Erin Collins, Helen Shapiro, Mark Hiller. Goucher College, 1021 Dulaney Valley Rd, Baltimore, MD.

The General Transcription Factors TFIIA and TFIID help situate RNA polymerase on the promoter of a gene during transcription initiation. TFIIA is made up of α, β, and γ subunits, and TFIID consists of TATA-Binding Protein (TBP) and fourteen TBP-associated factors (TAFs). TFIIA and TFIID physically interact with each other on promoters and may often work together during transcription initiation. *Drosophila melanogaster* may express two testis-specific homologs of the γ-subunit of TFIIA, γ-2a and γ-2b. These testis-specific homologs of the g subunit result from alternative splicing of the *tfiia-s-2* gene. *In vitro* expressed testis-specific γ-2a or γ-2b will associate with the widely expressed α and β subunits, indicating they may form TFIIA-like complexes. There are also several testis-specific TAF homologs known as tTAFs. A loss of function mutation in any of the tTAFs leads to a spermatocyte arrest phenotype, owing to a failure to transcribe many testis-specific genes that are normally expressed during the primary spermatocyte phase of spermatogenesis. There are no known *tfiia-s-2* mutants. We are investigating the possibility that the testis-specific *tfiia-s-2* gene products work with the tTAFs to activate testis-specific transcription.

605B Genome-wide analysis of the conserved transcription factor Grainy head reveals stable binding to target genes over development *Markus Nevil*[1], Eliana Bondra[1], Katharine Schulz[1], Tommy Kaplan[2], Melissa Harrison[1]. 1) Department of Biomolecular Chemistry, University of Wisconsin - Madison, Madison, WI; 2) School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel.

The Grainy head (GRH) transcription factor family is conserved from fungi to humans and has essential developmental functions in flies and mammals. *Drosophila* contain a single *grh* gene—the founding member of this family. By regulating gene expression, *Drosophila* GRH controls a variety of processes ranging from neuroblast differentiation to epithelial morphogenesis, and embryos lacking GRH die late during embryogenesis. Data suggest that GRH can function as both a transcriptional activator and repressor, but it is currently unclear how this dual functionality is controlled. To determine whether temporally distinct binding events allow GRH to control cell fate specification during development, we defined the transcriptional network regulated by GRH at multiple stages of embryonic development using a combination of ChIP-seq and RNA-seq. By identifying GRH-binding sites at various developmental time points, we determined that GRH binds thousands of sites that remain relatively stable over embryonic and larval development. Additionally, we identified a correlation between regions bound by GRH early in development and those with open chromatin later in development, suggesting that early GRH binding may function to enhance chromatin accessibility. By performing RNA-seq on embryos lacking either maternal or zygotic GRH, we defined the sets of genes that are activated and repressed by GRH and how they change over development. Despite the stable binding of GRH to target regions, our data reveal that GRH mediates expression of specific target genes at distinct specific developmental stages. Together these results reveal that GRH binds to target genes well before the GRH-dependent transcriptional program commences, suggesting it prepares the target locus for the subsequent recruitment of additional factors that execute stage-specific GRH functions.

606C The modERN Project - Genome-wide binding profiles of Transcription Factors *Alec Victorsen*[1], Lijia Ma[1], Matt Kirkey[1], Haneen Ammouri[1], Jeff Gersch[1], Cory Holgren[1], Michelle Kudron[3], Valerie Reinke[3], Dionne Vafeados[2], Sue Celniker[4], Bill Fisher[4], Soo Park[4], Bob Waterston[2], Kevin White[1]. 1) University of Chicago, Chicago, IL; 2) University of Washington, Seattle, WA; 3) Yale University, New Haven, CT; 4) University of California Berkeley, Berkeley, CA.

The goal of the modERN project is to continue the modENCODE project's work of characterizing regulatory networks in model organisms. A key component of gene regulation is through the interaction of sequence-specific Transcription Factor proteins (TF) and genomic regulatory elements. The locations of these elements in the genome can be found by Chromatin-Immunoprecipitation followed by NextGen Sequencing (ChIP-seq). The availability of suitable antibodies remains a significant barrier to large scale ChIP-seq experiments. To circumvent this issue, the modERN project has created transgenic *D. melanogaster* and *C. elegans* lines expressing tagged TFs, allowing a single tag-specific antibody to be used across multiple experiments. Furthermore these lines express the tagged TFs at near endogenous levels with correct spatiotemporal patterning, and are capable of rescuing null mutants. Using this strategy, the modERN project has successfully generated 300 transgenic *D. melanogaster* lines (available at the BDSC), 200 *C. elegans* lines (distributed by the CGC) and over 150 TF ChIP-seq datasets in both organisms. The breadth of data we have generated has yielded new and interesting results pertaining to both ChIP-seq as well as gene-regulation.

607A Mutational Analysis of the *Drosophila* Pol II CTD *Feiyue Lu*[1,2], Bede Portz[1], David Gilmour[1]. 1) BMB Department, Penn State University, University Park, PA; 2) The Huck Institutes of Life Sciences, Penn State University, University Park, PA.

The carboxyl-terminal domain (CTD) of the largest RNA polymerase II (Pol II) subunit, Rpb1, is a key player in eukaryotic gene regulation. One conserved feature in the CTDs of the higher eukaryotes is the presence of the consensus heptads $Y_1S_2P_3T_4S_5P_6S_7$ as well as the non-consensus heptads that are divergent from the consensus at one or more positions. The functions of the non-consensus heptads are largely unknown. Here, we use the *Drosophila* CTD as a model to investigate the function of the CTD in vivo. By testing various CTD-mutant forms of Rpb1 for their capacity to rescue lethality caused by endogenous Rpb1 depletion, we have identified a region containing the consensus heptads being essential for *Drosophila* development. This region encompasses sequences with the greatest homology to the human CTD. Remarkably, the human CTD, a CTD variant that contains a different assembly of non-consensus heptads, can partially function in place of the *Drosophila* CTD. Immunofluorescence analyses of polytene chromosomes from salivary glands reveal that an Rpb1 derivative with a CTD deletion of up to half the length of the *Drosophila* CTD associates with the chromosomes yet a CTD-less Pol II does not. To characterize the effects of the CTD mutations on transcription, we aim to compare the genome-wide distributions of mutant CTD Pol IIs with a wild-type Pol II. This work will establish a basis for examining CTD function in the context of a multicellular organism.

608B Age-related transcription changes in photoreceptor neurons are light-dependent Hana Hall[1], Jingqun Ma[1], Xinping Chen[1], Patrick Medina[2], Jeremiah Rounds[2], Rebecca Doerge[2,4], Don Ready[3], *Vikki Weake*[1]. 1) Biochemistry, Purdue University, West Lafayette, IN; 2) Statistics, Purdue University, West Lafayette, IN; 3) Biological Sciences, Purdue University, West Lafayette, IN; 4) Statistics, Carnegie Mellon University, Pittsburgh, PA.

Epigenetic mechanisms have been proposed to play key roles in the pathogenesis of ocular diseases associated with aging, such as diabetic retinopathy and age-related macular degeneration. Chromatin undergoes significant changes during aging and the pathogenesis of ocular disease; these epigenetic changes correlate with, and contribute to, misregulation of gene expression. An unanswered question is whether age-related changes in chromatin and gene expression drive the transition from aging to early disease state in ocular diseases of aging. Long-lived photoreceptor neurons might be uniquely vulnerable to the effects of aging because they must maintain the expression of genes important for the survival as well as cellular function throughout the adult lifespan. To characterize the mechanisms involved in age-dependent changes in gene expression, we sought to characterize the transcriptome of aging photoreceptor neurons in *Drosophila*. To identify genes that change expression profiles upon aging, we labeled and isolated nuclei from adult photoreceptor neurons and examined the transcriptome using RNA-seq at five time-points between 10 and 40 days post-eclosion. Using this approach, we identified 1200 genes with age-dependent changes in expression profiles. Genes that are upregulated with age are functionally-enriched for GO terms involved in stress-response and protein synthesis, whereas genes that are downregulated with age are enriched for GO terms such as ion transport, cell adhesion and neuronal function. Strikingly, the downregulated genes show maximal expression changes at the earliest time points examined, while the upregulated genes change later in life. This suggests that defects in transcription activation precede and may contribute to the increased stress response and cellular dysfunction in aging photoreceptor neurons. Since light is known to lead to increased oxidative stress in the eye, we tested the contribution of light to the age-dependent changes in gene expression. Notably, exposure of young flies to blue light induces similar changes in gene expression in photoreceptors to those that occur

during aging, consistent with the hypothesis that the age-related changes in photoreceptor gene expression are dependent on light. Thus, blue light provides us with an experimental system to identify the epigenetic mechanisms involved in the transcriptional decline observed during photoreceptor aging.

609C Deciphering the mechanisms of maternal versus zygotic Zelda *Danielle Hamm*, Kelsey Marshall, Eliana Bondra, Melissa Harrison. Department of Biomolecular Chemistry, University of Wisconsin - Madison.

In all animals, the genome is transcriptionally silent during the earliest stages of development while maternally deposited proteins and RNAs control cellular function. Within hours these maternal products are degraded, and the zygotic genome is robustly transcribed. This switch in developmental control, from maternal products to the zygote's own genome, is known as the maternal-to-zygotic transition (MZT). The transcription factor Zelda (ZLD) plays an essential role in global activation of the zygotic genome during the MZT in *Drosophila melanogaster*. ZLD is both maternally deposited and zygotically expressed. ZLD is a large protein with six C_2H_2 zinc fingers but no additional identifiable domains or predicted enzymatic activity that might offer clues as to its function. Furthermore, short ZLD isoforms, reported to be expressed in late-stage embryos and larvae, contain only the activation domain but without the DNA-binding domain. To further elucidate the function of ZLD, we generated isoform-specific deletions as well as point mutations in highly conserved protein domains using the CRISPR-Cas9 system. We show that the short isoforms of ZLD are dispensable for viability, and that the full-length isoform is both necessary and sufficient for animal development. Additionally, we find that while males with mutations in one of the N-terminal zinc fingers of ZLD are viable and fertile, these mutations cause maternal-effect lethality. This phenotype provides the first evidence for a maternal-specific role for ZLD. We are continuing to explore the regulatory function of this conserved protein domain. Our studies reveal that while both maternal and zygotic ZLD are required for viability, the essential functions of ZLD may vary throughout embryogenesis. Further dissection of individual protein domains will likely demarcate the unique stage-specific functions of ZLD necessary for development.

610A A dual kinetic switch underlies the function of the Drosophila Hox transcription factor Sex combs reduced *Dimitrios Papadopoulos*[1], Vladana Vukojevic[2], Lars Terenius[2], Rudolf Rigler[3], Pavel Tomancak[1]. 1) Max-Planck Institute of Cell Biology and Genetics, Dresden, Saxony, Germany; 2) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 3) Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

While Hox-Extradenticle (Exd) complexes represent a major functional entity of most Hox transcription factors, formation of higher-order Hox complexes on the DNA is required for at least part of their homeotic function in vivo.

We investigated the relative contributions of Scr homodimers and Scr-Exd heterodimers in Scr function, using genetic analysis and imaging methods with single-molecule sensitivity: advanced Fluorescence Imaging, Fluorescence Correlation Spectroscopy (FCS), Fluorescence Recovery After Photobleaching (FRAP) and Bimolecular Fluorescence Complementation (BiFC).

We uncovered marked differences in the DNA-binding kinetics between Scr-Exd heterodimers and Scr homodimers. While heterodimers form very fast on the DNA, have low binding affinities and result in short-lived transient complexes (with fast recoveries in BiFC-FRAP experiments), homodimers exhibit slower DNA-binding kinetics, but the complexes bind on the DNA long and strongly. Differences in the relative concentrations of Scr and Exd result in pronounced biases towards hetero- or homodimerization among genetically identical cells of the same tissue.

To further understand the functional significance of this kinetic regulatory mechanism, we have examined the requirement of homo- and heterodimers in the activation of three (and the repression of two) target genes during the induction of Scr-mediated homeotic transformations in the embryo and larval imaginal discs. This has been done by varying the genetic dosage of Scr and Exd. We found that transcriptional activation is favoured by Scr-Exd heterodimers, whereas Scr homodimers function as preferential repressors of Scr target genes. Finally, addition of Exd allows a constitutively inactive phosphomimicking form of Scr to become a functional transcriptional activator, capable of inducing homeotic transformations.

Taken together, our results unravel a novel mechanism of Scr-mediated gene regulation. Exd preferentially allows the rapid, transient formation of activation complexes on the DNA, whereas the homodimeric form of Scr acts as a preferentially repressive species with longer lasting DNA-binding properties. Our data also suggest that it is the protein-protein interactions within the homodimeric complex that account for the stronger binding of homodimers on the DNA.

611B *Drosophila* Nora Virus ORF1 Protein is Localized to the Nucleus *Larissa Attema*, Kimberly Carlson, Brad Ericson. Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a novel RNA picorna-like virus that has positive-sense, single-stranded RNA. Its mode of transmission is horizontal via the fecal-oral route. The viral genome consists of four open reading frames, two that specify structural proteins (ORF3 and ORF4), one encodes a replicase cassette (ORF2) and the 5' most ORF

encodes an RNAi inhibitor (ORF1). Nuclear localization signals (NLS) are sequences of amino acids that direct the transport of proteins into the nucleus of a cell. They can be monopartite or bipartite. Through a sequence analysis of the Nora virus gene ORF1, we discovered a putative bipartite nuclear localization signal. To verify that this NLS was transporting ORF1 protein into the nucleus, we constructed an ORF1-GFP fusion by isolating ORF1 and inserting it upstream of EGFP in the p-EGFP N3 plasmid, fusing ORF1 and GFP together. We then transfected this ORF1-GFP construct into S2 cells and observed the results by fluorescence microscopy. Our results suggested nuclear localization as the ORF1-GFP fusion protein staining merged with DAPI staining in the nuclei of the transfected S2 cells. We subsequently created mutants of ORF1 to eliminate the NLS. We analyzed them by again transfecting these constructs into S2 cells and observing the results with fluorescence microscopy. Elimination of the NLS in our mutants resulted in GFP staining in the cytoplasm of the cell instead of in the nucleus. DAPI staining of the same cells failed to show an merging of the staining. To our knowledge, this is the first example of an RNA virus that specifies an RNAi inhibitor that translocates to the nucleus. 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.

612C A heterodimer-based regulatory role for *Drosophila* truncated dADAR protein isoform function *Fatemeh Kohram*, Sushmita Ghosh, George Gruenhagen, Jack Vaughn. 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 embryo-specific truncated (TR). FL contains the deaminase catalytic domain and TR does not. 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. It has been shown that *in vitro* synthesized FL isoform is catalytically active, but is nearly completely inactive *in vivo* during embryo development. The mechanism(s) for this inactivity are incompletely known, and nothing is known about the function of the TR isoform. 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 x 10^6 year divergence time, suggesting a conserved function for this 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. Our current research centers on testing a model in which heterodimers between the two major isoform classes negatively regulate dADAR catalytic activity during embryo development in *Drosophila*. We have started testing this model by showing that the TR isoform can form a heterodimer with two known FL isoforms (FL3/4 and FL3a). For this purpose, we chose the yeast two-hybrid system to show interactions between these proteins. DNA clones for these isoforms were purchased from the DGRC. DNAs from these clones were inserted into yeast vectors for transformation into *S. cerevisiae* strains Y2HGold and Y187. Six combinations of yeast strains were made both by co-transformation and mating (FL3/4+FL3/4, FL3/4+FL3a, FL3/4+TR, FL3a+FL3a+, FL3a+TR, TR+TR). Y2HGold cells were also co-transformed with either pGBKT7-53+pGADT7-T or pGBKT7-lam+pGADT7-T as positive and negative controls, respectively. Yeast cells were grown on triple drop out media (TDO+X+A) lacking histidine, leucine and tryptophan plus Aurobasidin A with 200 ng/ml final concentration and X-a-Gal with 20 mg/ml final concentration and double dropout media (DDO+X) lacking tryptophan and leucine plus X-a-Gal with 20 mg/ml final concentration. All yeast cells grew on both media showing blue colonies except for the negative control strain which only grew on DDO+X media with white colonies. Our results show, for the first time, that the TR isoform protein can bind to both FL3/4 and FL3a isoforms. The resulting heterodimers are predicted to result in virtual absence of RNA editing during fly embryo development, as observed *in vivo*.

613A Broadly-expressed repressors integrate patterning across two embryonic axes through temporal control of enhancer action *Theodora Koromila*, Angelike Stathopoulos. Division of Biology and Biological Engineering, 1200 East California Boulevard, Pasadena, CA 91125 USA.

Patterning of embryos is accomplished through the combinatorial action of transcription factors, many having spatially-localized expression domains, but how broadly-expressed, often ubiquitous factors support gene expression is less well understood. Most studies of patterning mechanisms have focused on the combinatorial regulation of gene expression through joint action of activators and repressors, in which expression is support by Boolean integration of these positive and negative inputs. However, more recent studies in *Drosophila* have found evidence that broadly-expressed activators also contribute to patterning in non-linear ways. For instance, the maternally-deposited activator Zelda impacts patterning globally throughout the embryo influencing both AP and DV patterning. Here our analysis shows that the broadly-expressed repressors Runt and Suppressor of Hairless [Su(H)] act widely to support patterning throughout the *Drosophila* embryo. Previous studies in the *Drosophila* embryo have linked the repressors Runt and Su(H) to patterning of the anterior-posterior (AP) axis or dorsal-ventral (DV) axis, respectively. Our data show that, in addition to Runt, Su(H) also functions as a spatially-localized repressor to define anterior boundaries for a subset of genes along the AP axis. While in the trunk, where both are broadly expressed, we find

that Runt and Su(H) also influence spatiotemporal gene expression by controlling the timing of action of particular enhancers that drive expression along both AP and DV axes. These studies define a role for broadly-expressed repressors in the regulation of temporal action of enhancers, generally throughout the *Drosophila* embryo across both AP and DV axes, and thereby identify a novel mechanism for integrating spatiotemporal patterning information throughout the entire embryo.

614B Control of Hox transcription factor concentration and cell-to-cell variability by an auto-regulatory switch *Dimitrios K. Papadopoulos*[1], Kassiani Skouloudaki[1], Ylva Engström[2], Lars Terenius[3], Rudolf Rigler[4,5], Vladana Vukojevic[3], Pavel Tomancak[1]. 1) Max-Planck Institute of Cell Biology and Genetics, Dresden, Saxony, Germany; 2) Department of Molecular Biosciences, the Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; 3) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 4) Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden; 5) Laboratory of Biomedical Optics, Swiss Federal Institute of Technology, Lausanne, Switzerland.

Concentration and DNA-binding kinetics of transcription factors (TFs) are crucial determinants of their function in developing tissues. However, how their molecular numbers and cell-to-cell variability are controlled to ensure regulatory robustness remains poorly understood.

Using Fluorescence Correlation Spectroscopy (FCS), we have characterized the number of molecules and cell-to-cell variability of 14 endogenously tagged *Drosophila* TFs in imaginal discs. We found TF numbers ranging from few hundreds to almost ten thousand molecules per nucleus (28 nM to 1.1 μM). Variation in concentration of different TFs among neighboring cells increased with increasing average concentrations. Of all 14 TFs examined, Antennapedia (Antp) was found to display the highest concentration-to-variation ratio in third instar imaginal discs, whereas the opposite was observed in second instar discs. This suggested the presence of a developmental switch from a low concentration/high variation to a high concentration/low variation state. Using *Antp* transcriptional reporters in temporally resolved gain- and loss-of-function studies we have uncovered that Antp is sufficient and required to drive a developmental regulatory switch from a state of auto-activation to a state of auto-repression. This mechanism accounts for increase of concentration and suppression of inter-cellular variability in developing discs.

Our results underline the strength of FCS, coupled to endogenous gene-editing methodologies, to study molecular numbers and variation of protein concentration in developing tissue. They also support a dynamic mechanism by which Antp controls its own protein levels and variation during development.

615C Maternal-effect RNAi screen for genes that affect enhancer activity in the *Drosophila* embryo *Ashley Albright*[1], Elizabeth Roeske[1,2], Michael Eisen[1,2]. 1) Molecular and Cellular Biology, The University of California at Berkeley; 2) Howard Hughes Medical Institute, University of California Berkeley.

Enhancers drive patterned gene expression during development, but we have a poor understanding of how they work. Previous studies from our lab suggested that, in addition to patterning transcription factors that modulate the activity of specific enhancers, there is a parallel system that determines where enhancers are located in the genome and shapes the maturation of chromatin during early development to facilitate their activity. One factor involved in this parallel system in *Drosophila melanogaster* is the maternally-deposited Zelda (ZLD) that binds to sites destined to be enhancers prior to the onset of zygotic transcription and triggers a cascade of events including the deposition of histone acetyl and methyl marks characteristic of active enhancers. To better understand the specification of enhancer identity and activity in the early *D. melanogaster* embryo, we are performing a maternal-effect RNAi screen of histone modifiers, insulators, and mediators. Female flies containing one of two maternal Gal4 driver lines that act during oogenesis are crossed to male Transgenic RNAi Project (TRiP) shRNA lines. Embryos in the F2 generation are examined for overall morphological changes as well as expression of a patterned gene (*evenskipped*) and a ubiquitous gene (*sryα*). Using this dual-probe *in situ* approach for both *evenskipped* and *sryα*, we are able to determine whether any potential defects as a result of knocking down maternal mRNAs are due to aberrant transcription or enhancer-specific malfunctions. We have found several interesting candidates that have abnormal *eve* patterning, yet normal *sryα* expression. Preliminary evidence suggests that some chromatin modifiers, which may or may not act at enhancer regions specifically, modulate enhancer activity without affecting transcription as a whole.

616A Using live imaging of mRNA synthesis to explore selection on enhancer activity in *D. melanogaster* embryos *Augusto Berrocal*[1], Hernán García[1], Michael Eisen[1,2]. 1) Department of Molecular & Cell Biology, University of California, Berkeley; 2) Howard Hughes Medical Institute.

I am using live imaging methods that capture the precise temporal and spatial dynamics of enhancer driven transcription in *Drosophila melanogaster* embryos to study how selection tunes transcription during development. I have generated a BAC based reporter system consisting of the *evenskipped* locus, in which the *eve* gene has been replaced with a reporter containing an array of MS2 stem loops. In the presence of an MS2 binding protein fused to a fluorescent reporter, individual bursts of mRNA synthesis from this locus can be visualized by confocal microscopy

and quantified. I have modified the *eve* stripe 2 enhancer to remove binding sites for the repressors Giant (GT) and/or Kruppel (KR), which produce anterior (GT) and posterior (KR) expansions of the stripe, and am comparing the transcriptional dynamics (bursting frequency, duration, and intensity as well as the fraction of transcriptionally active nuclei) within the eve stripe and in the ectopic domains to ask whether enhancers are essentially switches that turn transcription on in the appropriate time and place, or if transcriptional dynamics are tuned more precisely, in which case we expect to see differences between nuclei within stripe 2 and those in the ectopic domains produced by the removal of repressor binding sites.

617B Modeling Enhancer Evolution in the *Drosophila montium* Species Subgroup *Michael Bronski*[1], Michael Eisen[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA.

Enhancers – cis-regulatory sequences that control transcription at a distance – play central roles in development, disease, and evolution. Enhancers integrate information encoded in the activity levels of transcription factors (TFs) to produce novel outputs through interactions between TFs and their binding sites (TFBSs) in the enhancer. However, the principles that relate the number, strength, and arrangement of TFBSs to the activity and output of an enhancer are still unclear. For example, the *eve* stripe 2 enhancer in *Drosophila* contains functional TFBSs for at least six TFs, and the removal of sites for any of these TFs affects enhancer output. Interestingly, the specific composition and arrangement of TFBSs in *eve* stripe 2 is highly variable across species, yet these altered enhancers drive identical patterns in the *D. melanogaster* embryo. We are seeking to exploit this phenomenon to better understand the evolutionary and functional constraints on TFBSs in *Drosophila* enhancers by developing accurate models of the process of binding site turnover that scrambles TFBSs. Towards this end, we sequenced and assembled the genomes of 12 closely related species in the highly speciose *Drosophila montium* species subgroup. By comparing dozens of enhancers across a large number of closely related species, we can study the earliest stages of enhancer divergence and observe individual changes in TFBSs. The median scaffold NG50 for our draft assemblies was approximately 51 kb, and our assemblies contain high percentages of genes and known enhancers. Preliminary analysis shows that binding site conservation is correlated with proximity to neighboring sites, and that new binding sites tend to overlap existing sites. Changes in the strength of highly conserved repressors (either strengthening or weakening) tend to be associated with gains of overlapping sites for the general regulatory Zelda sites. Finally, the loss of a repressor within a highly conserved cluster of binding sites can be compensated by the creation of a similar cluster of sites elsewhere in the enhancer, such that the proximate compensatory mutation can actually be the gain of an activator site. We are currently in the process of sequencing and assembling 15 additional *monitum* genomes. When these assemblies are complete, we will continue to systemically catalogue permissible TFBS changes across dozens of enhancers, identify compensatory mutations, infer changes that are forbidden, refine our model of enhancer evolution, and ultimately test our model by expressing synthetic enhancer constructs in vivo.

618C A relay mechanism between the K50 homeodomain proteins Bcd and Otd controls temporal and spatial patterning of distinct target genes in anterior regions of the Drosophila embryo *Rhea Datta*[1], Jia Ling[1], Timothy Bishop[1], Martha Bulyk[2], Stephen Small[1]. 1) Department of Biology, New York University, New York, NY; 2) Division of Genetics, Brigham and Women's Hospital & Harvard Medical School, Cambridge, MA.

The K50 homeodomain (HD) proteins Bicoid (Bcd) and Orthodenticle (Otd) bind to similar DNA sequences *in vitro*, and are expressed in patterns that overlap in time and space. Here we use chromatin immunoprecipitation experiments to show that the two proteins bind to overlapping and distinct genomic regions and a gene replacement assay to show that Otd is incapable of providing almost all Bcd-like functions *in vivo*. HD swaps between the two proteins show that the Bcd HD can confer many Bcd-like transcriptional activities to the Otd protein, suggesting that subtle differences in DNA-binding preferences are critical for their distinct *in vivo* activities. Protein-binding microarray assays and sequence mining of Bcd- and Otd-bound peaks identified preferred DNA-binding motifs for each protein and motifs for two cofactors required for Bcd-dependent transcription. Reporter gene tests of these motifs suggest two independent modes of target gene regulation, a relay by which genes activated by Bcd are maintained by Otd, and a separate Bcd-independent mechanism by which Otd regulates a distinct set of target genes.

619A Regulation of sexually dimorphic pigmentation alleles in the *Drosophila montium* subgroup *Emily Delaney*, Artyom Kopp. Evolution and Ecology, University of California-Davis, Davis, CA.

Sex-limited color polymorphisms provide an excellent model for studying the origin of sexually dimorphic gene regulation because monomorphic and sexually dimorphic alleles segregate within the same species. In the *Drosophila montium* subgroup, *D. serrata* females have light or dark abdomens but males have only light abdomens—this female-limited pigmentation is controlled by a single autosomal locus with a dominant dark allele and a recessive light allele. We mapped female color to a short (< 1 kb) non-coding, structural variant in the first predicted intron of the transcription factor *POU domain motif 3* (*pdm3*) and found light and dark alleles that differ in size and sequence. The dark allele contains putative binding sites for the transcription factors *dsx* and *Abd-B*, providing a

potential mechanism for how *pdm3* might produce pigmentation in the posterior of females. We present data demonstrating how each allele at this structural variant influences the spatiotemporal expression and splicing of *pdm3* in both sexes and color morphs (light and dark). Our work identifies the molecular changes correlated with a shift from monomorphic to dimorphic alleles, providing insight into the first step in the evolution of sexual dimorphism.

620B Genomic dimensions of Su(H)-targeted regulatory belts in *Drosophila* Elizabeth Stroebele[1,2], *Timothy Fuqua*[1], Madelyn Warren[1], Albert Erives[1]. 1) Biology, The University of Iowa, Iowa City, IA; 2) Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Southwestern Medical Center, Dallas, TX.

Asymmetric Notch signaling promotes divergent fates in select cells throughout metazoan development. In the receiving cell, signaling results in cleavage of the Notch intracellular domain and its import into the nucleus, where it binds Suppressor of Hairless [Su(H)] to promote gene expression in conjunction with contextual cues in the surrounding DNA sequence. To investigate the nature of this contextual logic, we identify ~1,200 Su(H)-site containing regulatory belts that are conserved in *D. melanogaster* and *D. virilis.* Each of these Su(H)-type regulatory belt (SUHRB) is a 0.6–1.0 kb chain of conservation peaks consistent with a transcriptional enhancer or core promoter. These regulatory belts contain one or more canonical binding sites for Su(H) along with approximately 15–30 other binding sites. Interestingly, SUH-RBs are densely clustered in certain chromosomal regions such as the *E(spl)*-complex, the *WNT* gene complex, and genes encoding Notch receptor ligands (*Delta* and *Serrate*), suggesting a generic role for Notch signaling in gene regulatory networks involving these signaling factors. SUH-RBs overlap most known Su(H)/Notch-target enhancers and others, including non-embryonic enhancers that are not identified by embryonic ChIP-seq peaks. Thus, SUH-RBs overcome the stage-specific nature of embryonic ChIP-seq peaks and suggest a pervasive role for contextual tissue-specific pioneer and/or enhancer-licensing factors. SUH-RBs also delineate false positive ChIP-seq peaks, which do not overlap SUH-RBs, are missing even the weakest Su(H)-binding sequences, and have the shortest ChIP peak widths. Last, we characterize several novel enhancers including Su(H)-dependent enhancers at *Notch* and *Delta*, intestinal enhancers at *A2bp1* and *hedgehog*, and distinct enhancers at *roughest*, *E2f1*, and *escargot*.

621C *REDfly*: The Regulatory Element Database for *Drosophila* *Marc S. Halfon*[1,2,3], John Rivera[1,2], Mohammad Zia[1,2], Steven M. Gallo[1,2]. 1) University at Buffalo, Buffalo, NY; 2) NY State Center of Excellence in Bioinformatics & Life Sciences, Buffalo NY; 3) Roswell Park Cancer Institute, Buffalo, NY.

The REDfly database is a comprehensive source of data on *Drosophila cis*-regulatory sequences containing records for empirically validated *cis*-regulatory modules (CRMs, “enhancers”) and transcription factor binding sites (TFBSs) reported in the published literature. REDfly’s goal is to include all functionally tested sequences regardless of whether they have observable 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 approximately 700 publications and contains more than 22,000 records of reporter constructs regulating over 575 genes, including over 5600 “minimal” CRMs from transgenic in vivo reporter assays and over 10,000 from cell-culture assays, as well as over 2000 TFBSs. Extensive abilities exist for database searching and results filtering. The default search option now retrieves all annotated CRMs within a user-defined distance of a locus, not just those explicitly associated with a specific gene. Users can also choose whether to include cell-line results in their search output, or to focus only on records drawn from transgenic reporter assays. Additional improvements scheduled for release in 2017 include further improved search and download capabilities, addition of ChIP-derived TFBSs, predicted CRMs, integration with Galaxy, and substantial new curation. 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 and can be followed on Twitter at @REDfly_database.

622A Sequence determinants of enhancer-promoter specificity in a shared intergenic space *Tim Howes*[1,2], Michael Eisen[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley; 2) Howard Hughes Medical Institute.

Enhancers activate the transcription of nearby genes, but they do not indiscriminately activate all nearby genes. Instead, they often show specificity for a particular gene despite the presence of other proximal promoters. We are using engineered loci to understand the genomic features that cause an enhancer to associate with one promoter rather than another. We have modified BAC clones of regions from the *Drosophila melanogaster* genome that contain a pair of genes with a shared 5′ intergenic space that contains tissue-specific enhancers for each gene. We have modified the BACs to produce MS2 and PP7 tagged reporter-gene transcripts, and we are exchanging the positions of the enhancers to explore how the resulting expression patterns of the two genes are affected. We image enhancer activity in living embryos using fluorescently tagged MS2 and PP7 binding proteins, allowing for high precision spatial

and temporal characterization and comparison of expression patterns. In addition to testing the effects of enhancer position, we are also testing whether enhancer-promoter specificity depends on the presence of insulator sequences and/or particular transcription factor binding sites within the enhancers. By examining several such gene pairs, we hope to identify general sequence features that determine whether an enhancer will activate a particular gene.

623B Salivary gland specific DNA binding of CrebA *Dorothy Johnson*[1], Joslynn Lee[2], Michael Wells[1], Rebecca Fox[1], Matthew Slattery[2], Deborah Andrew[1]. 1) Department of Cell Biology, Johns Hopkins School Medicine, Baltimore, MD; 2) Department of Biomedical Sciences, University of Minnesota Medical School, Duluth, MN.

CrebA, the Drosophila Creb3/3L homologue, upregulates almost all known components of the canonical secretory pathway to increase secretory capacity of the salivary gland and other tissues whose major function is to produce high levels of secreted cargo. Microarray analysis has also demonstrated that CrebA increases the levels of transcripts encoding tissue-specific secreted cargo. As a first step toward learning how CrebA regulates both general and tissue-specific genes, we carried out tissue-specific ChIP-Seq analysis. Because CrebA is expressed in multiple tissues, we generated a set of tools to allow us to carry out salivary gland specific ChIP-Seq analysis. By expressing a functional UAS-CrebA-GFP under the control of either sage-Gal4 or fkh-Gal4 (two drivers whose expression overlaps in only the salivary gland), and isolating chromatin with αGFP followed by sequencing of the coprecipitated DNA, we were able to identify salivary gland-specific CrebA bound genes. We also performed a ChIP-seq analysis in wild type embryos using CrebA antiserum to gain insight into CrebA binding in the entire embryo, including salivary glands. Analysis of known binding motifs identified in salivary gland CrebA binding revealed an enrichment in the known binding sites for Creb3/3L family members. An analysis of GO terms of the predicted SG-specific CrebA bound genes revealed that, as expected, secretory pathway component genes are the major class of genes bound by CrebA. We also discovered an enrichment of target genes encoding nucleic acid binding proteins, including a gene known as Tudor-SN. Tudor-SN is a DNA/RNA binding protein that has been implicated in the stabilization of secretory mRNAs and its loss results in cuticle defects similar to those observed with loss of CrebA and of core secretory component genes. Future plans include more detailed analysis of CrebA binding sites and predicted targets, and a follow up characterization on the potential role of Tudor-SN in salivary gland secretion.

624C The co-activator, co-repressor, Su(H) concentration ratios are a major determinant of cell-specific Notch transcriptional outputs in *Drosophila.* *Y. Kuang*[1], O. Golan[2], R. Kovall[3], R. Kopan[1], D. Sprinzak[2], B. Gebelein[1]. 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Biochemistry and Molecular Biology, Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; 3) Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH.

Notch signaling is reiteratively used throughout development. In *Drosophila*, the Notch intracellular domain (NICD) enters the nucleus upon ligand induced proteolysis, and binds the transcription factor Suppressor of Hairless (Su(H)) to activate transcription. In the absence of Notch signals, Su(H) can bind the same DNA sites to repress transcription via co-repressor proteins (Hairless (H)). To define how DNA binding site architecture alters cell-specific transcription responses, we generated synthetic reporters containing two types of DNA sites: non-cooperative sites (referred to here as CSL (Cbf1/Su(H)/Lag1) sites) that bind individual Su(H)/NICD and/or H/Su(H) complexes, and Su(H) paired sites (SPS) that promote cooperative NICD interactions but non-cooperative H/Su(H) binding. By characterizing each reporter genes' transcriptional behavior in different tissues, we found that: 1) different tissues have distinct activating thresholds, 2) tissues vary in maximal reporter activity and 3) reporters show an unexpected bell-shaped transcription response curve with increasing Su(H) binding sites. Strikingly, we did not observe an expected maximal plateaued response as a function of binding site number, which is predicted if Su(H)/NICD occupancy is strongly favored over Su(H)/H binding. Instead, we found an unexpected bell-shaped response as a function of CSL and SPS site numbers. Overexpressing NICD strongly activates reporters with high copies of Su(H) binding sites, demonstrating that the reduction of transcription observed with higher copies of binding sites is not due to heterochromatin formation. The mathematical model that best fits this data describes Notch-dependent transcriptional responses as a competition between Su(H)/NICD (co-activator), Su(H)/H (co-repressor) and free Su(H) molecules for the same DNA sites. This model predicts that it is not the absolute number of NICD molecules alone that defines cell-specific outputs, but the ratio of co-activator, co-repressor and Su(H). We have already tested that, in larval wing discs, the expression of a synthetic Notch reporter is altered by changing Notch dosage. Future studies will test and refine this model by measuring reporter activity in animals with altered gene dosage of key Notch pathway genes that modify the coactivator/corepressor/Su(H) ratios.

625A An enhancer's length and composition are shaped by its regulatory task *Lily Li*, Zeba Wunderlich. Department of Developmental and Cell Biology, UCI, Irvine, CA.

Enhancers drive the spatiotemporal gene expression patterns required for virtually every biological process in metazoans. For example, during development, enhancers drive the expression patterns that specify cell fate

decisions. We propose that that enhancer architecture, in terms of enhancer length, number of transcription factor binding sites (TFBSs), and average information content, reflect the complexity of the enhancer's regulatory task. In the context of development, we define the complexity of a regulatory task as the number of cell fates specified in a set of homogeneous cells at one time. Thus, early in development when the cells are mostly homogeneous and are adopting many different cell fates, the complexity is high; in contrast, late in development when cells are making simple binary decisions between cell fates, the complexity is low. We hypothesize enhancers with more complex tasks will be longer, have more TFBSs, and lower average information content because increased numbers of TFBSs can be arranged in more ways, allowing for enhancers to drive distinct more patterns of expression. To test this hypothesis, we first compare ~100 enhancers driving the *Drosophila* anterior-posterior (AP) and dorsal-ventral (DV) patterning systems. These systems exemplify the disparity in decision complexity that enhancers need to drive, as the AP axis consists of 14 segments whereas the DV axis consists of six germ layers and sublayers. As expected, we find that the enhancers patterning the more complex AP axis are longer, have more TFBSs, and have lower average information content than the DV enhancers. We then consider a set of ~3500 developmental enhancers and characterize changes in the architecture of the enhancers active over embryogenesis. We find that enhancers active later in development are shorter than those active early in development. These results help explain why enhancer architecture is so diverse and imply that enhancer identification tools need to account for differences in enhancers' regulatory tasks to be effective in multiple systems.

626B Determinants of Transcriptional Enhancer Function in *D. melanogaster* Embryos *X.-Y. Li*[1], M. Eisen[1,2]. 1) Howard Hughes Med Inst, Univ California at Berkeley, Berkeley, CA; 2) Department of Molecular and Cell Biology, Department of Integrative Biology, QB3 Institute, University of California, Berkeley, CA.

Although we have known of the existence of transcriptional enhancers that regulate gene expression at a distance for over three decades, and that their activity is mediated by the interaction of sequence-specific transcription factors with binding sites within enhancers for nearly as long, the relationship between the binding site composition and organization of enhancers and their activity remains mysterious. Recently, studies from our lab and others have shown that a maternal factor, Zelda (ZLD), plays an important role in determining the location and mediating the function of enhancers in early *D. melanogaster* embryos. In the studies that followed we have investigated the effect of ZLD binding site mutations on a number of enhancers, and found that they often displayed only modest effect. In addition, insertion of ZLD sites into sequences that contain what appear to be appropriate numbers of binding sites for patterning factors like Bicoid is not sufficient to convert them into functional enhancers. These findings suggested that there are additional features of enhancers still to be discovered. We have therefore evaluated the effects of mutations in *eve* stripe 2 and *hb* distal anterior enhancers by randomizing the sequences between known transcription factor binding sites. Surprisingly, we have found that such changes dramatic decreased the activity of these two enhancers. Currently, we are carrying out experiments to further delineate the specific sequences that are important and investigate whether the observed effect was caused by changes in nucleosome organization.

627C Transcriptional dynamics of *trans-* vs. *cis*-acting enhancers in *Drosophila* *Grace C. McKenzie-Smith*[1], Nica N. Sabouni[2], Hernan G. Garcia[2], Jack R. Bateman[1]. 1) Biology, Bowdoin College, Brunswick, PA; 2) Physics, University of California Berkeley, Berkeley, CA.

Transcription requires interactions between an enhancer and a promoter. Enhancers are thought to usually act in *cis*, activating genes on the same chromosome as the enhancer itself. However, enhancers can also act in *trans*, activating a gene on a separate chromosome, a phenomenon known as transvection. Recent studies of transvection using fluorescent reporters suggest that relative levels of expression are stochastic and vary from cell to cell. However, the dynamics of activation are largely unexplored. To study this question, we are implementing the MS2/MCP system, which allows direct, real time visualization of transcription. MS2 RNA stem loops transcribed from a reporter transgene are bound by a GFP tagged MCP, creating fluorescent spots at sites of active transcription. We are currently testing enhancers active in the late embryo and eye disks for their capacity to drive MS2 reporters both in *cis* and in *trans.* This work will provide a basis to develop a model of enhancer transcriptional dynamics in *Drosophila.*

628A Dissecting intertwined enhancers driving distinct patterns of CNS-specific transcription for the divergent genes dCORL and twin of eyeless on chromosome 4 Nancy Tran, Norma Takaesu, *Stuart Newfeld*. Sch Life Sci, Arizona State Univ, Tempe, AZ.

CORL proteins (SKOR in mice and Fussel in humans) are a subfamily of CNS-specific nuclear factors related to Smad-interacting Sno/Ski oncogenes. However, their developmental roles remain largely unknown in all species. We previously showed that dCORL (fussel in Flybase) functions between the myoglianin receptor Baboon and ecdysone receptor-B1 expression in mushroom body neurons of third instar larval brains (Takaesu et al. Development 2012). To better understand dCORL CNS-specific regulation we generated a series of 11 reporter genes on chromosome 4 covering the 19kb dCORL locus and the 12kb region between dCORL and the divergently transcribed

gene twin of eyeless (toy). Toy is also a CNS-specific gene with an expression pattern completely distinct from dCORL. Examination of each reporter in triple labeling experiments with FISH for dCORL mRNA and antibodies to both lacZ and toy will reveal which reporters contain enhancers for each gene. Studies of embryos, larval CNS and adult brains will allow the tracking of reporter expression over time to determine if any enhancers switch from toy to dCORL and vice versa. The conservation of CNS-specific expression and amino acid sequence identity between dCORL and its mammalian homologs suggests that clues to the regulation of dCORL may suggest new hypotheses for the regulation of the mouse SKOR and human Fussel genes. Lastly, the association of human Fussel with ataxias that interrupt sleep suggests that a better understanding of dCORL regulation may shed light on the etiology of these poorly understood diseases.

629B Single Molecule Imaging of RNA polymerase and Transcription Factors in Developing *Drosophila* Embryos *Michael Stadler*[1], Mustafa Mir[1], Xavier Darzacq[1], Michael Eisen[1,2,3]. 1) MCB, UC Berkeley, San Francisco, CA; 2) Department of Integrative Biology; 3) Howard Hughes Medical Institute, University of California, Berkeley, USA.

Animal development is driven by programmed patterns of differential gene expression. Despite the importance of these processes, we do not yet have a clear picture of how transcription factors, enhancers, promoters, and RNA polymerase work together to produce robust transcriptional outputs. We have used single-molecule imaging of RNA polymerase II and developmental transcription factors in the early *Drosophila melanogaster* embryo to explore how transcription is controlled during animal development. We have developed methods for tagging endogenous proteins with either fluorescent proteins or the HaloTag, and have further adapted techniques to introduce HaloTag ligands conjugated with bright, photostable organic dyes to early embryos. Imaging these tagged proteins on a lattice light sheet microscope shows that tracking of single molecules is possible for long durations with high temporal resolution. We utilize these methods to characterize the dynamics of RNA polymerase II during activation of the zygotic genome and elucidate the role of sub-nuclear organization in transcription regulation.

630C Regulation of αFtz-F1 Pair-Rule Function by Transcriptional Corepressors *Alys M. Cheatle Jarvela*, Alicia Briscoe, Leslie Pick. Entomology, University of Maryland, College Park, MD.

Nuclear receptors are a family of transcription factors with a number of critical roles in embryonic development, human disease, and insect life-cycle progression; thus, understanding how their activity is controlled has important implications for both medicine and agriculture. During early *Drosophila* embryonic development, nuclear receptor αFtz-F1 helps to establish pair-rule patterning, which divides the embryo into functional segmental units (1, 2). αFtz-F1 is maternally deposited throughout the embryo, but its activity is limited by co-expression with Ftz, which is expressed in pair-rule stripes (1, 3). We are investigating whether there are additional post-transcriptional mechanisms in place to prevent αFtz-F1 activity in Ftz^- territories, especially protein-protein interactions with corepressors. We have taken two complimentary approaches towards identifying such corepressors. Using Crispr-Cas9 genome-editing technology, we are producing an αFtz-F1 allele with an endogenous FLAG tag to facilitate unbiased co-immunoprecipitation experiments. Additionally, we are using the extensive Transgenic RNAi Project (Harvard) resources in combination with a maternal-specific Gal4 system to screen a large number of candidate corepressors (4). Expression of αFtz-F1-dependent minimal enhancer-lacz reporters has allowed us to determine the effects of putative corepressor knock-down on αFtz-F1 activity. Using this approach, we have been able to both rule-out several potential corepressors known to interact with other nuclear receptors, and also identify Rpd3 (Hdac1) as a candidate for further investigation.

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631A Discrete regulatory domains control the expression of simple patterns during oogenesis *Nicole T. Revaitis*[1], Robert A. Marmion[1], Maira Farhat[2], Vesile Ekiz[2], Wei Wang[3], Nir Yakoby[1,2]. 1) Center for Computational and Integrative Biology, Rutgers, The State University of NJ, Camden, NJ; 2) Department of Biology, Rutgers, The State University of NJ, Camden, NJ 08103; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544.

Patterning of the *Drosophila melanogaster* eggshell has been extensively studied, however, the *cis*-regulatory modules (CRMs), that control the spatiotemporal expression of genes, are mostly unknown. This study takes advantage of the Flylight GAL4 collection, composed of over 7000 lines generated from intronic and intergenic

regions of DNA, and cross-listed the ~1200 genes that are contained within this collection to the 84 genes that are known to be expressed during oogenesis. There are 22 genes, or 281 lines, common between the two lists. Of these, 61 lines recapitulate the full or partial patterns of their endogenous gene. Using RNA-seq to identify isoforms expressed during oogenesis, we map the distribution of the 61 fragments to the corresponding gene model, and found an enrichment of fragments active in the first intron. In addition, we demonstrate the use of different anteriorly active FlyLight lines as tools to disrupt eggshell patterning in a targeted manner. Interestingly, while the average fragment is roughly 3 kB, there is usually only one component of the endogenous gene's pattern in each fragment. Our results further support that complex gene patterns are assembled by CRMs controlling simple patterns.

632B Roles of the *dA2BP1* in maintaining structural protein stoichiometry and fiber diversity of *Drosophila* muscles *KETAKI KAMBLE*. Indian Institute of Science, Bangalore, India.

Muscle is a highly heterogeneous and plastic tissue with multiple signaling pathways regulating different fiber profiles suitable for specific functional requirements across different species. *Drosophila* muscles can be divided into two fiber types – Fibrilar (Indirect Flight Muscles- IFMs) and Tubular (leg muscle, abdominal muscle, jump muscle etc.). Though these muscles have been characterized in different contexts with respect to their function, morphology and organization; the developmental processes that decide the fiber diversity is not well known. Recent studies show that, transcription factor Spalt major (*Salm*), under the regulation of homeodomain proteins- Extradenticle and Homeothorax, promotes fibrilar fate in IFMs. In the present study, we have identified roles of the *Drosophila* Ataxin 2 Binding Protein 1 (dA2BP1), a RNA binding protein, in dissipation of Salm mediated muscle fiber diversification process. A2BP1 is known to have a conserved RRM (RNA recognition motif) that binds to 5'-UGCAUG-3' sequences in RNA to regulate splicing. Here, we show that *Drosophila* A2BP1 is expressed throughout the major events of adult IFM development and it has variable distribution across muscle fiber types. Interestingly, *dA2BP1* knock down as well as over expression results in muscle hypercontraction phenotype in the IFMs. Our investigations show that the muscle hypercontraction phenotype is a byproduct of the combination of stoichiometric imbalance of structural proteins and lack of their proper isoforms. *dA2BP1* knockdown also shows severe defects in tubular muscles- TDT and lateral abdominal muscles. We found that dA2BP1 negatively regulates Arrest, a previously identified protein involved in fiber specific splicing. Arrest and dA2BP1 exhibited opposite distribution profiles across fiber types and genetic interaction studies between them reveal the importance of this differential distribution for specific splicing functions. Finally, we show that both dA2BP1 and Arrest carry out the fiber specific splicing functions under the regulation of the transcription factor, Salm.

633C Alcohol dynamically alters alternative splicing in the brain *Sarah Signor*[1], Jeremy Newman[2], Lauren McIntyre[2], Sergey Nuzhdin[1]. 1) Molecular and Computational Biology, University of Southern California, CA, USA; 2) Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, Florida, USA.

Alternative splicing expands the complexity of the transcriptome and plays a critical role in disease and development. Available evidence suggests that it is an important part of the response to drugs of abuse, and potentially the changes that lead from abuse to addiction. There has been a handful of studies on alternative splicing in response to alcohol, but very little is currently known about the role of splicing. Here we investigate dynamic changes in alternative splicing in *Drosophila* over the course of an exposure to alcohol by sequencing the head transcriptome of 6 genotypes of *Drosophila* for 10, 20, and 30 minutes after exposure. We find that even on this time scale there are significant changes in the representation of different isoforms. This includes many genes involved in homeostatic synaptic plasticity. Changes in homeostatic synaptic plasticity have been suggested to be the underlying cause of addiction to alcohol. While there are known to be functional differences between a few isoforms of these genes, this area is largely unexplored and their relationship to alcohol exposure is unknown. This suggests that changes in splicing may be an important aspect of the response to alcohol abuse and the development of addiction.

634A Translational regulation of *cyclin B* during G2 prophase of meiosis in *Drosophila* males *Catherine Baker*, Margaret Fuller. Dept Developmental Biol, Stanford Univ Sch Medicine, Stanford, CA.

A unique feature of germ cell development is the delay in progression through G2/M of meiosis I. In *Drosophila* males, meiotic G2 prophase lasts 3.5 days and allows for a 25-fold increase in cell volume and the transcription of over 1000 genes needed for post-meiotic differentiation. A critical question is how the developmental program in spermatocytes is coordinated with the core cell machinery to enact this prolonged G2 prophase. One such core cell component is Cyclin B, which is subject to dynamic translational control during spermatocyte development. Cyclin B protein levels, while high in spermatogonia, are low in immature spermatocytes and high again in mature spermatocytes poised to enter the meiotic divisions. The RNA-binding protein Rbp4 and its cofactor Fest are required for translational repression of *cycB* in immature spermatocytes, and this effect is mediated by sequences in the *cycB* 3'UTR. Activation of *cycB* translation in mature spermatocytes requires Syncrip (Syp),

the *Drosophila* homolog of mammalian hnRNP Q. Syp physically interacts with Rbp4 and Fest in both immature and mature spermatocytes, and Syp (like Rbp4) binds the *cycB* RNA, though the Rbp4-Syp interaction is RNA-independent. Motivated by preliminary data from IP-mass spectrometry experiments, we are currently investigating whether both Rbp4/Fest-mediated repression and Syp-mediated activation of *cycB* translation might regulate the activity of the core translation initiation complex.

635B Engineering Genetic Tools to Study *Drosophila* Development *Thomas Jacobsen*, Ashley Jermusyk, Chase Beisel, Gregory Reeves. Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

Tools for regulating gene expression have been widely developed for single-celled organisms, such as bacteria and yeast. However, these genetic tools are currently limited or have yet to be established in more complex organisms.

Here we describe a potential gene regulatory tool to be adapted in *Drosophila melanogaster*: self-cleaving hammerhead ribozymes. Self-cleaving ribozymes are natural RNA structures that can undergo a phosphodiester cleavage reaction, resulting in mRNA cleavage and degradation. To implement fine-tuning capabilities for this genetic tool, various “competing sequences” can be cloned upstream of the ribozyme. Depending on thermodynamics, these “competing sequences” can interact with a major stem of the ribozyme through Watson-Crick base pairing, thus allowing for various conformational changes of the ribozyme structure. Since the cleavage activity of the ribozyme is strongly dependent on the kinetics of the cleavage reaction, as well as the thermodynamics of the “competing sequences”, the level of gene expression can be predicted and tuned using various sequences differing in length, as well as base-pair complementation.

Multiple ribozyme constructs were cloned either upstream or downstream of *gfp*, which was expressed from a constitutive promoter. These constructs were transiently transfected into HEK293T cells. The addition of different “competing sequences” resulted in variable expression of GFP, but also appeared to drastically reduce its translational activity. Future work will be conducted to analyze the thermodynamics of the “competing sequences”, as well as to incorporate these tools to study a synthetic gene network mimicking the endogenous Bicoid/*hunchback* network in *Drosophila*.

636C The mechanism of transcriptional activation by two different transcriptional activators through a single binding site *Koichi Miyagawa*, Kazutaka Akagi, Yusuke Takakura, Hitoshi Ueda. Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

At the onset of metamorphosis, ecdysteroid secretion induces many gene expression including transcription factors. *Ecdysone-dependent gene 84A*, *Edg84A*, encoding pupal cuticle protein is one of these gene and is expressed in high level during the mid- to late prepupal period. *Edg84A* is activated by two transcription factors, DHR3 and βFTZ-F1. DHR3 is expressed during the late larval to mid-prepupal period induced by 20-hydroxy ecdysone, and activates the *βftz-f1* gene and βFTZ-F1 is expressed during the mid- to late prepupal period. *Edg84A* promoter region has a single element to bind both DHR3 and βFTZ-F1, but they can not bind at the same time. To investigate the regulation mechanism of the *Edg84A* gene by two transcriptional activators, we established transgenic fly lines carrying the *Edg84A* promoter- *LacZ* fusion gene which have a mutation recognized only by either DHR3 and βFTZ-F1. These transgenes are expressed the reporter gene but the expression levels were decreased compared to the control transgene, suggesting that both factors can activate the *Edg84A* gene but both factors are necessary for the full level activation. Furthermore, ChIP assay revealed that βFTZ-F1 dose not bind efficiently to the *Edg84A* promoter carrying mutation which can not bind DHR3. We also analyzed the histone modification states of the transgene by ChIP assay. The result revealed that H3K4me3 level was increased after puparium formation in the promoter recognized by DHR3 but that level was not increased in the promoter which can not recognized by DHR3. From these results, we expect that DHR3 induces change in chromatin structure and this facilitates the βFTZ-F1 binding to the promoter for the high level expression of the *Edg84A* gene.

637A Characterization of a Possible IRES Site in the Nora Virus Genome *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). This is in contrast to other picornaviruses that have one long ORF. The coding potentials of the ORFs are reasonably well characterized. ORF3 and 4 are known to code for the capsid proteins and ORF1 codes for an RNAi inhibitor. Between ORF3 and ORF4 there are 85 nucleotides of non-coding RNA, which may act as an internal ribosome entry site (IRES) for the translation of ORF4. However, this region is not obviously related to any known IRES sequences. To test this hypothesis, we designed multiple RT-PCR primers that flank ORF3 and ORF4, and the IRES region. The results suggest that subgenomic RNAs are not being produced, but studies are underway to further characterize this region. GFP constructs designed to test the IRES potential of the non-coding region between ORF3 and ORF4 are currently being evaluated. RT-PCR reactions indicate that subgenomic RNAs are not being produced. Currently, plasmid constructs containing ORF3, the putative IRES sequence, and GFP immediately downstream of

the IRES are being evaluated in S2 cells to further characterize this region. Preliminary results indicate internal ribosome initiation at the IRES. 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.

638B A feedback role for *Drosophila* RNP-4F protein in regulation of its own translation during development *Divya Soundararajan*, Sushmita Ghosh, Girija Lakshmi, Jack Vaughn. Biology, Miami University, Oxford, OH.

In eukaryotes, intron splicing plays a major role among mechanisms of gene expression control and also in the creation of diversity among encoded proteins. Defects in splicing have been shown to be a major cause of many human diseases. Although the mechanism behind splicing is well known, regulation of splicing factor protein expression is poorly understood. The *Drosophila rnp-4f* gene encodes RNP-4F protein, which is a splicing assembly factor that acts as a chaperone to unwind the U6-snRNA helical stem prior to its dimerization with U4-snRNA during spliceosome assembly. We have recently proposed a model for the molecular mechanism behind gene expression control of *Drosophila rnp-4f*. Using overexpression of RNP-4F in transgenic fly embryos, it was found that *rnp-4f* translation is enhanced as indicated by the GFP reporter used. Previous work had shown that in an RNA electrophoretic mobility shift assay (REMSA), radiolabeled *in vitro* transcribed *rnp-4f* 5'-UTR stem-loop RNA mobility was diminished in the presence of embryo protein extract to form two shifted bands. Since the smaller band disappeared when proteins were from *dADAR* null mutant embryos, but the mobility of the larger band was not affected, it was concluded that dADAR protein binds to the stem-loop. It was suspected that RNP-4F was the other protein, but there was no *rnp-4f* null mutant line for use in testing identity of the protein in the larger band. We therefore combined the conventional REMSA method with western blot technique, and found that stem-loop RNA binds to RNP-4F protein, as shown by the anti-RNP-4F antibody used. Further, developmental westerns show a peak in RNP-4F protein in very early embryos, followed by a decline at the MBT stage, then a second peak at 8-12 h. This result is qualitatively the same as the profile shown by conventional developmental REMSA, supporting the model that RNP-4F protein plays a feedback role in regulation of translation during development by binding to the 5'-UTR stem-loop of its own mRNA.

639C Uncovering a Role for Nucleoporin Megator as a Nuclear Scaffold Component *Jennifer Aleman*, Maya Capelson. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.

The nuclear pore complex (NPC) is a large macromolecular structure embedded in the nuclear envelope and is well known for its role in selective transport between the nucleus and cytoplasm. The NPC consists of ~30 Nucleoporin proteins or Nups. Beyond their structural transport role, Nups have recently been shown to have a role in genome organization and transcriptional regulation. These gene regulatory events occur during *Drosophila melanogaster* development. The details and the mechanisms of this regulation and organization are not well understood. Our lab has characterized a nuclear structure formed by specific Nups. We refer to this structure as the nuclear scaffold or nuclear cables since this accurately describes the localization of the Nups – binding in large interspersed domains along chromatin, as observed by immunofluorescence staining of polytene chromosomes of larval salivary glands. One of the key components is a nuclear basket Nup, Megator (Mtor or TPR in mammalian cells), which has been implicated in both transcriptional regulation and transport functions of the nuclear pore. The goal of my research is to define the biological role of the Mtor-defined nuclear scaffold. In determining the role of the nuclear scaffold we project to also define the gene regulatory role of Mtor. We have analyzed Mtor's role in transcriptional response to ecdysone hormone and have found that it appears to be required for proper transcriptional regulation of select target genes. We also conducted salivary whole mount immunoflourescence experiments to define the function of the Mtor-formed nuclear scaffold structure in import and chromatin binding of a developmental transcription factor, ecdysone receptor. Our future plans include assessing whether the Mtor-defined nuclear scaffold plays a role in transcriptional regulation by means of gene loop formation as has been shown for other Nups, as well as identifying the mechanism by which Mtor contributes to ecdysone-induced transcriptional response.

640A Disruption of X Chromosome Suppression in *Drosophila melanogaster* Males Lacking a Y Chromosome *Kathleen E Gordon*, Colin D Meiklejohn. School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

Sex chromosome-specific gene regulation in the male germline has evolved repeatedly in taxa with XY heteromorphic sex chromosomes. In mice, epigenetic modification of both the X and Y chromosomes in meiotic spermatocytes results in a 10-fold decrease in expression of 80% of X-linked genes, termed meiotic sex chromosome inactivation (MSCI). Despite the existence of MSCI in mammals, nematodes, and grasshoppers, X-chromosome regulation is poorly understood in the *Drosophila* male germline. Prior studies have failed to show the extreme down-regulation of X-linked genes during spermatogenesis seen in other taxa. However, testis-specific transgene reporters show lower expression at X-linked insertions than autosomal insertions. To identify genetic factors involved in X-

suppression, we focused on the role of the Y chromosome in X-linked gene expression in the male germline. We used two transgene reporter constructs, carrying either either *lacZ* or *GFP*, driven by the promoter of the testis-specific gene *ocnus*, which robustly shows X-suppression. Expression from each construct was assayed from four X-linked insertions and four autosomal insertions. We generated XO males carrying X-linked and autosomal transgene insertions using three different strains carrying compound-X/compound X-Y sex chromosomes and used qPCR to measure expression of *lacZ* and *GFP* mRNA in XO and XY males. Our results confirm significant lower expression from X-linked insertions relative to autosomal insertions in XY males for both reporters (X-suppression). We find a significant statistical interaction between the location of the transgene insertion (X-linked *vs.* autosomal) and the presence/absence of a Y chromosome, due to a reduction in the magnitude of the difference between X-linked and autosomal insertions in XO males. In some cases, X-linked insertions show increased expression in XO males relative to XY males; in other cases, autosomal insertions show decreased expression in XO males relative to XY males. Further studies will elucidate how the Y chromosome participates in X chromosome regulation and how the absence of a Y chromosome disrupts X-suppression.

641B The *Drosophila* Lysine-specific Demethylase 2, dKdm2, is not Required for Normal Development Yani Zheng[1], Xingjie Ren[2], Xinsheng Gao[1], Jian-Quan Ni[2], *Jun-Yuan Ji*[1]. 1) Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX; 2) Gene Regulatory Laboratory, School of Medicine, Tsinghua University, Beijing 100084, China.

Post-translational modification of histones plays important roles in regulating chromatin dynamics and transcription in eukaryotes. The status of histone methylation is controlled by histone methyltransferases and histone demethylases. Misregulation of histone methylation can not only lead to aberrant gene expression and abnormal development, but also contribute to diseases such as cancer. Studies of the mammalian lysine-specific demethylase 2 (KDM2) homologs, KDM2A and KDM2B, suggest they can be either tumor suppressive or oncogenic, depending on specific biological contexts, which is consistent with the view that histone modification enzymes play context-specific roles in regulating tumorigenesis. However, the role of KDM2s during development in the whole organisms remains poorly understood. Unlike vertebrates, *Drosophila* has only one KDM2 homolog (dKdm2), but its utility as a model system has been controversial because its roles in regulating development were unknown due to the poor quality of mutant alleles available. We generated three deficiency lines that disrupt the *dKdm2* gene, and performed an exhaustive genetic analysis of *dKdm2* mutant alleles available. Our genetic, molecular and biochemical analyses suggest that *dKdm2* is not essential for viability and fertility. We further validated this conclusion by generating additional *dKdm2* null alleles using the CRISPR/Cas9 technique. We have observed that the *dKdm2* homozygous null animals are fully viable and fertile with no developmental defects observed under laboratory conditions. Taken together, our genetic, molecular, and biochemical analyses demonstrate that the *dKdm2* gene is not required for *Drosophila* development.

642C Genome transcriptional activity under stress in *Drosophila melanogaster*: the role of the *limk1* gene *Ekaterina Nikitina*[1,2], Svetlana Gorohova[2], Anna Medvedeva[2], Elena Savvateeva-Popova[2]. 1) anatomy and physiology, Herzen State Pedagogical University, St-Petersburg, Russian Federation; 2) neurogenetics, Pavlov Institute of Physiology RAS, St-Petersburg, Russian Federation.

Neurodegenerative diseases are caused by a complex interaction of unfavorable external factors and specific genome features that predispose to the development of a disease. *Drosophila* constitutes a convenient model for studying the link between genome organization and chromosome architecture observed in cognitive disorders. Insufficiently studied stress factor are weakened magnetic fields. We analyzed the influence of weakened by shielding geomagnetic field on transcriptional activity of the genome in *Dr. melanogaster*. Under stress we observed the dependence of transcriptional activity modification on the structure of a gene LIMK1, the key enzyme of actin remodeling cascade. The analysis of histone methylation has revealed the high level of a transcription in both stocks - *Berlin* (control) and *agn*^{ts3} (defect of *limk1* gene) – in normal conditions. However, we have found significant suppression of a transcription after impact of weak static magnetic field in both stocks, especially at *agn*^{ts3} mutant. Thus, the change of transcriptional activity in the conditions of weakening a geomagnetic field can be caused by disturbances of actin remodeling cascade.

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643A The Activation of Cryptic *lawc* Gene Promoter by Small dsRNA in *Drosophila* S2 Cells R. Cherezov, J. Vorontsova, *O. Simonova*. Lab of Regulation of morphogenesis, Koltzov Institute of Developmental Biology, Moscow, Russian Federation.

The activation of gene expression by small dsRNAs that target gene promoters (RNAa) is considered to be an evolutionary conserved mechanism that was described in human, rat, mouse, *Petunia* and *C. elegans*. Recently we described RNAa-like phenomenon in *Drosophila* (Cherezov et al., 2013). The increase of *lawc* mRNA gene expression in response to treatment with long dsRNA complimentary to *lawc* ORF was detected

in *D. melanogaster* S2 cell culture also. Using Northern blotting, 3'RACE and 5'RLM-RACE we showed that after the transfection of long dsRNA the pattern of *lawc* gene expression changed and the new short *lawc* transcripts were activated. We proposed the existence of a cryptic transcriptional regulatory element within the *lawc* ORF. In order to test this possibility we used ElemeNT software (Sloutskin et al., 2015) and found predicted TATA-less promoter region that matched with the Bridge element and Initiator located adjacent to it in a strict spacing requirement within *lawc* ORF. We tested the functional significance of predicted regulatory element combination using Luciferase reporter system. The fragment of *lawc* ORF was cloned into the promoterless/enhancerless luciferase reporter vector pGl4.10 Luc2 (Promega) upstream of Luciferase ORF. Than *Drosophila* Schneider 2 (S2) cells or Human embryonic kidney 293 (HEK293) cells were transfected with constructed plasmid. We detected a significant amount of luciferase activity in both types of cells while cells transfected with the empty pGl4.10 Luc2 vector displayed almost no luciferase activity. Furthermore using 5'RLM-RACE we detected new transcriptional start site within *lawc* ORF that coincided with the predicted one.To examine the necessary of Ago1 and Ago2 proteins (members of the Argonaute (AGO) protein family required for processing of long dsRNA) for *lawc* RNAa we used RNAi-mediated knock-down of *ago1* and *ago2*. As a result the effect of *lawc* RNAa in cells depleted of Ago1 or Ago2 was significantly decreased. *Drosophila* AGO2 mediates siRNA-guided endonucleolytic cleavage of mRNAs, whereas *Drosophila* AGO1 plays a role in mRNA decay triggered by miRNAs. Thus *lawc* mRNA activation is mediated by small dsRNA complimentary to *lawc* ORF.

In conclusion, our results clearly demonstrate the existence of functional cryptic promoter in *lawc* ORF that is probably not active under normal conditions but could be activated by small dsRNA in *Drosophila* S2 cells.This study was funded by Russian Foundation for Basic Research (research projects No. 16-04-00829-a and 16-34-00296-mol_a).

644B Regulation of Transcript Levels During the Maternal to Zygotic Transition Across Species of *Drosophila* *Emily Cartwright*, Susan E. Lott. Evolution and Ecology, University of California, Davis, Davis, CA.

The maternal to zygotic transition (MZT) occurs during the early embryogenesis of all animals, whereby control of development is transferred from maternally deposited factors, such as proteins and mRNAs, to the zygotic genome. The handoff from maternal to zygotic control during the MZT is a conserved, highly regulated process that is necessary for survival and proper development of the organism. While the MZT occurs during the early development of all animals, there are differences in the pools of mRNAs that are deposited by mothers and transcribed by zygotes across species. Prior work in the Lott lab has found that there are significant differences in which mRNAs are maternally deposited and transcribed from the zygotic genome in the early embryo between closely related species of *Drosophila*. While the MZT has been studied in a number of model systems, gene regulatory changes that lead to differences in mRNA pools between species have not been identified. In order to study how this highly regulated process evolves, we examined early embryogenesis using three closely related species of *Drosophila* (*D. simulans*, *D. sechellia*, and *D. mauritiana*). We utilized genetic crosses and backcrosses, and conducted allele-specific RNA-sequencing of single embryos, to identify changes in *cis* and in *trans* regulatory elements that cause differences in maternal deposition of mRNAs, degradation of these maternal mRNAs, and transcription of early zygotic genes between these species. Through this study, we identified a greater number of changes in *cis* than in *trans* regulatory elements across species, genome-wide, indicating that gene regulation during early embryogenesis, a time of high regulatory constraint, may evolve more readily through alterations in *cis* regulatory elements. We can now characterize the molecular basis of identified regulatory changes, which can help us understand how regulation works genome-wide during each critical stage of the MZT. Further, these studies will allow us to identify the functional consequences of alterations in gene regulation through analysis of single genes that have changed across species.

645C Model Organism Encyclopedia of Regulatory Networks (modERN) *Susan Celniker*[1], Kevin White[2], Mark Gerstein[3], Valerie Reinke[4], Robert Waterston[5], The modERN Consortium. 1) Berkeley Dros Genome Ctr, Lawrence Berkeley National Lab, Berkeley, CA; 2) Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL; 3) Computational Biology and Bioinformatics Program, Yale University, New Haven, CT; 4) Department of Genetics, Yale University School of Medicine, New Haven, CT; 5) Department of Genome Sciences, University of Washington, Seattle, Washington.

Our goal is to create comprehensive maps of transcription factor (TF) binding sites in *D. melanogaster* and *C. elegans.* We have developed an effective high-throughput pipeline to create strains of flies and worms with GFP-tagged TFs, which we use to perform ChIP-seq experiments. We are targeting 708 sequence specific TF genes in the fly and 725 TF genes in the worm (excluding 234 nuclear hormone receptor genes likely to be *C. elegans* specific with a role in environmental sensing). Including lines generated previously for the modENCODE project, we now have 403 fly and 327 worm GFP tagged TF lines. We have used these lines to perform 253 ChIP-seq experiments in the fly and 160 experiments in the worm. For a limited number of transcription factors, we also are conducting RNA-seq of strains with knock down of specific transcription factors by deletion or RNAi to identify

potential target genes of the given TF and to validate targets identified by our ChIP-seq experiments. To date, we have collected RNA-seq data from 19 worm TF knockdown lines and 14 TF fly knock down lines. RNA-seq data and ChIP-seq data are being deposited to the ENCODE DCC for access by the research community. TF tagged lines available through the Bloomington Drosophila Stock Center and Caenorhabditis Genetics Center.

646A A Comprehensive *Drosophila* melanogaster Transcription Factor Interactome *Leila Shokri*[1], Antonina Iagovitina[2], Korneel Hens[3,4], Riccardo Dainese[3], Stephen Gisselbrecht[1], Sachi Inukai[1], Johannes Bischof[5], Edy Furger[5], Konrad Basler[5], Bart Deplancke[3], Martha Bulyk[1,6]. 1) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; 2) Systems Biology Graduate Program, Harvard University, Cambridge, MA, USA; 3) Laboratory of Systems Biology and Genetics, Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne; Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4) University of Oxford, Neural Circuits and Behaviour, OX1 3SR Oxford, United Kingdom; 5) Institute of Molecular Life Sciences, University of Zurich, CH-8057 Zurich, Switzerland; 6) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

Combinatorial interactions among transcription factors (TFs) play essential roles in generating gene expression specificity and diversity in metazoans. Here we present a yeast two-hybrid (Y2H)-based TF-TF interactome among nearly complete *Drosophila* TF repertoire that are engaged in ~2000 interactions. Orthogonal *in vitro* (e.g. microfluidics) and *in vivo* (e.g. BiFC) analyses validated many of these interactions. Our *in vivo* studies suggested functional interactions and allowed us to propose biological functions for TFs lacking previous experimental annotation. Given the importance of combinatorial TF interactions in gene regulation, we integrated *in vitro* protein binding microarray (PBM) data with publicly available *in vivo* DNA binding data from genome-wide chromatin immunoprecipitation (ChIP). We uncovered cases of TF recruitment to DNA via an interacting TF that may be a general mechanism, by which TFs regulate additional sets of target genes, aside from binding directly to their own DNA recognition sites. This network represents, to our knowledge, the most comprehensive and integrated resource for TF interactions for *Drosophila*.

647B The *ab initio* design of sythetic sequences driving expression of *even-skipped* stripe 2 *Kenneth Barr*[1], AhRam Kim[1], Carlos Martinez[1], Jennifer Moran[2], Kevin White[2], John Reinitz[1]. 1) Ecology and Evolution, University of Chicago, Chicago, IL; 2) Human Genetics, University of Chicago, Chicago, IL.

The regulatory DNA driving the expression of the *Drosophila* developmental gene *even-skipped* is perhaps the best studied regulatory region in all of metazoan biology. Cellular-resolution data on protein and mRNA levels during *Drosophila* embryogenesis has allowed for quantitative modeling of transcriptional control at single nucleotide precision. Such models represent a mathematical formulation of decades of experimental results and have been successful in describing the behavior of enhancer fusions and predicting the expression driven by enhancers not included in training data. If these models capture every aspect necessary for enhancer function, their use can be extented to the design of entirely sythetic DNA elements capable of driving expression in arbitrary patterns during development. To this end we designed two synthetic sequences that we predicted would drive expression overlapping the second stripe of *even-skipped*. These sequences contained binding sites for each transcription factor known to be required within the native *eve* stripe 2 enhancer (MSE2), yet neither sequence drove detectable expression when assayed *in vivo*. To determine what was missing, we designed a set of 20 synthetic enhancers that represent a neutral evolutionary path between our synthetic sequences and MSE2. Sequences with close homology to MSE2 drove expression as expected, while those that did not drive the predicted pattern revealed hidden constraints in cis-regulatory logic.

648C Measuring the Influence of *cis*-Acting Changes on the Transcriptional Response to Infection in *Drosophila melanogaster* *Bryan A Ramirez-corona*, Zeba Wunderlich. Developmental and Cell Biology , UC Irvine, Irvine, CA.

Drosophila and other insects respond to bacterial infections by differentially regulating the expression of hundred through the action of several signaling pathways. This response to infection is highly variable from one individual to the next. Past efforts to uncover the genetic underpinnings of this variability have focused on protein-coding regions. However, there is ample variation in the non-coding DNA sequences of *Drosophila melanogaster*, and in other contexts, this variation has been shown to drive phenotypic differences between individuals.

Therefore, there is a need to measure how sequence variation in regulatory regions affects complex transcriptional response to infection. To measure the contribution of *cis*-regulatory sequences to transcriptional variation, we generated F1 hybrids by crossing two highly inbred founder strains from the *Drosophila* Synthetic Population Resource. Three hours after inoculating the animals with Gram-negative bacteria, we measured gene expression in both the F1 hybrids and parental strains. By comparing the expression levels of each parental allele in the F1 hybrid to expression in the parental strains, we can identify genes whose differential expression is caused by changed in *trans* or changes in *cis.* We expect to find many genes with changes in *cis*-regulatory regions that account for

changes in transcriptional response to immune stimulation. Furthermore, we expect that these differences will be a first step in understanding the underlying syntax and architecture of these regulatory regions.

649A Interchromosomal pairing and regulation of a stochastically expressed gene in the fly retina *Kayla Viets*, Robert Johnston. Department of Biology, Johns Hopkins University, Baltimore, MD.

How nuclear architecture influences gene regulation is poorly understood. One of the first described examples of interchromosomal interactions, transvection, involves two mutant alleles interacting via homologous chromosome pairing to regulate gene expression in flies. A transvection-like mechanism called Interchromosomal Communication (InterCom) upregulates and downregulates the on/off expression frequency of the stochastically expressed gene *spineless* (*ss*) in the *Drosophila* retina. Here, we identify a biological role for InterCom in averaging the on:off ratio of naturally derived *ss* alleles to direct retinal patterning. InterCom requires copies of *ss* to be in physical proximity ("gene pairing"), but pairing is not sufficient for crosstalk between alleles. ~100-kb transgenes containing *ss* drive gene pairing between non-homologous chromosomes and endogenous *ss*, but similarly sized transgenes neighboring *ss* cannot drive gene pairing. Our data are consistent with a model in which strongly interacting "super-pairers" such as *ss* button chromosomes together to facilitate cross-regulation in *trans*, rather than chromosomes zippering evenly along their entire lengths.

650B The role of Hox-cofactor interactions in vivo *Siqian Feng*, Richard Mann. Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY.

Hox proteins specify segmental identities along the anterior posterior body axis in metazoans. How Hox proteins specify the morphology of different body segments presents a paradox: Different Hox proteins display characteristic in vivo functions, yet have very similar DNA binding profiles as monomers in vitro. One hypothesis to explain this paradox is that Hox proteins interact with cofactors, and different Hox-cofactor complexes bind to unique DNA sequences. Indeed, it has been shown previously that Extradenticle (Exd) is a common cofactor for all 8 *Drosophila* Hox proteins, and different Hox-Exd heterodimers show distinct DNA binding preferences in vitro. However, the extent to which Hox-DNA binding and function is modulated by Exd in vivo is still unknown. To address this question, we have engineered the endogenous Hox genes *Sex combs reduced (Scr)* and *Ultrabithorax* (*Ubx*) to create 3xFLAG tagged alleles and 3xFLAG tagged (YPWM-AAAA) alleles, which have the canonical Exd interaction motifs mutated. We have characterized the phenotypes of YPWM-mutated alleles in embryos, imaginal discs and adults, and compared the DNA binding profiles of wild type and YPWM mutant Hox proteins by ChIP-seq. Our results represent an important step towards understanding how cofactors modulate the functions and binding specificities of Hox proteins *in vivo*.

651C Optogenetic activation of gene transcription with spatiotemporal resolution *L. De Mena*, P. Rizk, C. Cruz, Y. Zhang, D. Rincón-Limas. Neurology, University of Florida, Gainesville, FL.

Tools that enable manipulation or perturbation of gene function are critical to define its contribution to normal development and disease. Thus, advances in our capacity to design and use novel strategies for controlling gene expression at fine spatial and temporal resolution will be essential for deciphering many cellular, developmental and physiological processes. Unfortunately, current inducible expression systems involve steroid hormones, antibiotics, heavy metals, or heat shock, which can induce toxicity or pleiotropic effects. What if transgene expression could be rapidly activated and immediately reversed with a switch triggered by light? Optogenetics exploits the advantages of photoactivation, which provide speedy response, spatial precision and reversibility. Here we present the implementation of two genetically encoded light-dependent expression systems in Drosophila cells (S2R) coupled with the **UAS-GAL4** and **LexAop-LexA** systems. The **phytochrome**-based system (PhotoGal4), possesses all the elements required to induce transcription of genes under the GAL4 promoter upon **red light** stimulation. The PhyB-PIF3 interaction involves splitting the DNA binding domain of Gal4 from its activation domain which must interact to trigger gene transcription of the target gene. On the other hand, the **LOV domain**-dependent system (LANS) controls transcription of genes under LexA promoter through **blue light** stimulation. Upon blue light treatment, the LANS domain undergoes a conformational change that exposes the NLS allowing translocation of the system to the nuclei where it promotes transcription. Our hypothesis is that a combination of both systems will serve as a high-resolution device to sculpt gene expression in *Drosophila* with agile on-off control and with extraordinary precision and resolution.

652A The genomic mechanisms of transcriptome turnover in Drosophila *Ammon Thompson*, Artyom Kopp, Michael May, Brian Moore, Li Zhao, David Begun. Ecology and Evolution, UC Davis, Davis, CA.

One of the central questions in evolutionary genetics concerns the relative contribution of structural and regulatory changes to phenotypic evolution. Do organ transcriptomes gain and lose genes mainly through structural changes such as gene duplication and deletion or is regulatory change the principle mechanism? Quantifying the relative contribution of these myriad mutations to the mode and tempo of transcriptome turnover will shed light on the genetic

basis of organ evolution.

To quantify and compare these rates, we generated RNA-seq data from the male testes and accessory glands of eleven species in the Drosophila melanogaster species group. These organs are an ideal model as both are under strong sexual selective pressures and experience rapid rates of evolution. We developed an analytical framework for calculating the probability that a gene has gained or lost expression in each organ of each species. Combining expression and genomic data and using evolutionary analysis we were able to make inferences about the relative importance of myriad structural and regulatory mutations to transcriptome turnover.

Our data show that the mode or pattern of transcriptome turnover is similar between the organs but the rate of testes turnover is greater. Interestingly, we also found transcription factor cooption from other tissues throughout the melanogaster group yielding lineage-specific expression in each tissue. Transcription factor expression divergence occurred at a faster rate in the testes. These patterns suggest transcriptome turnover may be in part driven by shifts in the expression of upstream regulators. This work gives a genome level view of the evolutionary dynamics of transcriptome turnover and organ evolution.

653B Dissecting the gut-microbiota-brain connection in Drosophila *Scott Keith*[1], Rory Eutsey[1], Stacie Oliver[1], Malachi Blundon[1], Brad Solomon[2], Heewook Lee[2], Carl Kingsford[2], N. Luisa Hiller[1], Brooke McCartney[1]. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Computational Biology Department, Carnegie Mellon University, Pittsburgh, PA.

The microbiota–the populations of bacteria, fungi, archaea, and viruses living symbiotically in and on animal hosts–affect many aspects of host physiology. Surprisingly, recent evidence suggests that the microbes occupying the mammalian gut can impact neurological functions that alter learning, memory, and behavior. However, the mechanisms by which gut microorganisms affect animal neural physiology and behavior are poorly understood. *Drosophila melanogaster* provides a powerful system to study bacterial-animal symbioses. The goal of this work is to discover molecular mechanisms underlying the connections between the bacterial gut microbiota, animal brains, and behavior, using *Drosophila* as a model system. To achieve this goal, we are taking a combined “bottom-up” and “top-down approach”, focusing on the community architecture of the gut microbiota and microbial-dependent changes in neural gene expression, respectively. We used a combination of culture-dependent and culture-independent techniques to study the composition and spatial distribution of gut bacteria in a recently established wild type line, Top Banana (TB). Sequencing of 16S rDNA and standard culturing methods revealed an *Acetobacter* dominant bacterial community in the TB gut, consistent with previously reported bacterial communities in other wild-type *Drosophila*. Fluorescent *in situ* hybridization (FISH) against 16S rRNA using genus-specific probes suggests an uneven distribution of bacterial taxa throughout the gut. To investigate the microbiota’s effects on host behavior, we used RNA-seq to identify genes that are differentially transcribed in the heads of axenic (AX) flies, those without a microbiota. This transcriptomic comparison revealed 343 candidate genes with altered expression in AX fly heads. Further analyses of these candidate genes via Nanostring nCounter transcript analysis, RT-qPCR, and FISH will identify robust, microbe-dependent transcriptional changes, and the specific cell types in which they occur. The identities of these genes will inform future hypotheses testing the behavioral consequences of observed expression differences, and the extent of host brain regulation by microbes. We will then determine the particular bacterial taxa responsible for these gene expression phenotypes, and their potential behavioral correlates, using fly cultures monoassociated with particular *Acetobacter* and *Lactobacillus* species. Identifying these microbiota-influenced genes will provide mechanistic clues into the molecular pathways linking symbiotic microbes to host neural function and behavior.

654C Studying heterochromatin formation in Drosophila using single embryo ChIP *Carolus Chan*, Shivani Mahajan, Doris Bachtrog. Integrative Biology, UC Berkeley, Berkeley, CA.

Chromatin Immunoprecipitation Sequencing (ChIP-seq) has given much insight into the transcription factor binding sites and chromatin landscape. Specifically, using ChIP to study chromatin in Drosophila embryonic development often requires collecting embryos over the course of hours followed by formaldehyde crossing linking and sonication. This often requires large amount of starting material which makes studying subtle changes in chromatin at specific stages of Drosophila embryogenesis difficult. In addition, differences in chromatin states between sexes cannot be discerned. This makes it difficult to study how heterochromatin formation or dosage compensation happens in embryogenesis especially in Drosophila with neo-sex chromosomes. Here, we use an ultra low input – native ChIP to study heterochromatin formation at specific stages of embryogenesis. Starting material can go as low as a single embryo allowing us to analysis differences in heterochromatin between sexes in embryogenesis.

655A The Fbox protein CG6758 regulates LaminB to suppress Xbp1 induced cell death in the Drosophila eye *Pedro Domingos*, Cristiana Santos, Nadine Schweizer, Catarina Gaspar, Vanya Rasheva, Fátima Cairrão. ITQB-UNL, Oeiras, Portugal.

The unfolded protein response (UPR) is composed by homeostatic signaling pathways that are activated by

excessive protein misfolding in the endoplasmic reticulum (ER). Ire1 signaling is an important mediator of the UPR, leading to the activation of the transcription factor Xbp1. Prolonged ER stress and UPR activation may lead to cell death and photoreceptor degeneration. Our aim is to identify genes important for photoreceptor degeneration induced by ER stress. We found that over-expression of activated Xbp1^{spliced} induces retinal degeneration in *Drosophila* and we performed a genetic screen to identify genes that, downstream of Xbp1^{spliced}, mediate the induction of retinal degeneration. In this genetic screen, we used the FLPase/FRT technique and looked for EMS induced mutations that suppress Xbp1^{spliced} induced retinal degeneration. So far, we identified mutations in 3 independent genes that are able to suppress Xbp1^{spliced} induced retinal degeneration: Xpd, Eaf and CG6758. We are pursuing CG6758, a gene encoding an Fbox protein with unknown biological function. F-box proteins form complexes with Skp, Cullin-1 and E2 ubiquitin ligases (SCF complexes) to mediate the ubiquitination of specific substrates, and leading to the degradation of these substrates by the proteasome. We did a proteomic screen to identify binding partners of Fbox/CG6758 and identified LaminB as a candidate substrate of Fbox/CG6758. We are currently investigating how regulation of LaminB protein levels by Fbox/CG6758 leads to the suppression the retinal degeneration process induced by Xbp1^{spliced}.

656B ATAC-seq on sectioned *D. melanogaster* embryos reveals spatially uniform chromatin *Jenna Haines*[1], Michael Eisen[1,2]. 1) Molecular and Cellular Bio, University of California Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, University of California Berkeley.

As the *Drosophila* embryo transitions from the use of maternal RNAs to zygotic transcription, domains of “open” chromatin, with relatively low nucleosome density and specific histone marks, are established at promoters and enhancers involved in patterned embryonic transcription. An open question, however, is whether these chromatin domains are themselves spatially patterned or if patterned activity occurs on spatially uniform chromatin. To address this question, we have probed chromatin accessibility across the anterior-posterior axis of early *Drosophila melanogaster* embryos by applying a transposon based assay for chromatin accessibility (ATAC-seq) to the anterior and posterior halves of hand-dissected, cellular blastoderm embryos of *Drosophila melanogaster*. Preliminary findings suggest that for most regions identified as active in intact embryos there is little difference between anterior and posterior halves. This suggests that for many enhancers, chromatin accessibility is not a primary regulator of transcription factor binding and that other regulatory elements must be responsible for selective transcription factor binding. However, our study identified several specific enhancers that are more accessible in the embryo half where they are functionally active and are less accessible where they are not functionally active. This observation has important consequences for how we think about both the mechanisms by which domains of chromatin accessibility are established and how patterned activity arises during development.

657C Enhancer priming and decommissioning in the two major germ layers during embryonic cell fate decisions *James P Reddington*[1], David Garfield[1,2], Darren Cusanovich[3], Charles Girardot[1], Aslihan Karabacak[4], Uwe Ohler[4], Jay Shendure[3], Eileen Furlong[1]. 1) Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany; 2) IRI Life Sciences, Humboldt-Universität zu Berlin, Germany; 3) University of Washington, Seattle, WA, USA; 4) Max Delbrück Center, Berlin, Germany.

During embryonic development cells undergo successive restriction of cell-fate that culminates in the diversity of differentiated cell-types observed in a complex multicellular organism. Transcriptional enhancers are central to this process as they establish differential patterns of gene expression among otherwise equal cells. How distinct sets of enhancers are selected in each cell lineage is not completely understood. To address this issue in the context of a developing organism, we are utilizing two complementary approaches that overcome the challenges posed by cellular heterogeneity. Firstly, we combined flow cytometry of formaldehyde-fixed cell nuclei with DNaseI-seq to measure genome-wide chromatin accessibility in pure populations of embryonic cells. We used this method to map regulatory element usage during key transitions in the development of mesoderm and neural cell lineages. We identify ~20,000 distal regulatory elements, including 85% of all known *Drosophila* enhancers to date, in addition to ~16,000 new putative enhancers, over half of which were not identified in DNaseI-seq experiments performed on whole-embryos. We show that patterns of lineage-specific chromatin accessibility are established early in development, and that lineage progression is associated with a high turnover of regulatory elements, mostly at sites distal from gene promoters. We are using machine-learning approaches to find informative motifs related to regulatory elements with different developmental properties, allowing us to identify the key *trans*-acting factors involved in establishing lineage-specific enhancer usage. By integrating information on *in vivo* tested enhancer activity we address the relationship between the timing of enhancer accessibility and functional activity during development. In parallel, we have performed the first single-cell assays of chromatin accessibility on a developing organism. Here we show that embryonic cells cluster primarily by germ-layer of origin. We identify genomic elements that display cell-cluster-specific accessibility, and show that this information is sufficient to predict tissue-specific enhancer activity *de*

novo. Overall, this work broadens our understanding of how cis-regulatory networks drive cell-fate decisions, and takes us one step further towards a systems level understanding of transcriptional regulation during development.

658A SAGA complex modularity and chromatin interactions *George Dialynas*[1], Shiyuan Chen[1], Susan Abmayr[1,2], Jerry Workman[1]. 1) Stowers Institute For Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

The SAGA complex is a major transcriptional co-activator, which 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 activators. The general structure and function of SAGA complex 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 and individual modules may play unique roles in different tissues or cell types. The modular SAGA complex has activities that include acetyltransferase (HAT), deubiquitinase (DUB) and co-activator functions. All subunits are not required for each activity, and each activity may be differentially required for cell-type specific gene expression. Therefore we use Drosophila to address: 1) The role of SAGA subunits/modules in the recruitment of SAGA to promoters (do SAGA subunits function independently or do they work in a cooperative manner for SAGA recruitment), 2) The role of different SAGA modules in regulating transcription at individual genes, or even at different sets of genes (function vs. recruitment). We have effectively knocked-down the SAGA complex subunits of the two enzymatic modules (GCN5, Sgf29, nonstop, Ataxin 7) which was followed by immunofluorescence analysis on salivary gland polytene chromosomes. This analyses shows that both enzymatic modules/activities are essential for organismal viability (lethality occurs at late larval stages) and our morphological analyses shows that these two modules disrupt SAGA complex binding on chromatin, yet the modules are not interdependent for their binding on chromatin. Currently we extend this analyses with co-IP experiments to determine the impact of each module on the SAGA complex structural intergrity. To further dissect the role of the enzymatic SAGA modules in transcription and development we performed whole genome RNA-seq profiling which shows that more than 2000 genes are misregulated upon loss of one HAT or DUB module subunit that fall into 30 different subsets, showing that SAGA modules regulate different gene subsets. These results suggest that SAGA modules are differentially required in development. We are coupling these analyses with whole genome ChIP-seq analyses to identify which genes of these subsets are directly regulated by the SAGA modules.

659B *Chd1* as a modifier of position effect variegation *P. Tram Bui*, Lakshmi Bugga, Jennifer Armstrong. Claremont McKenna College, Pitzer College, Scripps College, CA.

Chromodomain helicase DNA binding protein 1 (CHD1) is a conserved chromatin remodeling and assembly factor implicated in the regulation of nucleosome dynamics at transcriptionally active genes. We observed that increased levels of CHD1 result in loss of HP1a binding on chromosomes, despite different localization patterns of the two proteins. Given the role of CHD1 in chromatin structure at active genes, and its ability to antagonize binding of heterochromatin proteins, we wondered whether loss of function *Chd1* alleles could function as enhancers of variegation [*E(var)*] of the mosaic expression of euchromatic genes placed near or within heterochromatin. To our knowledge, *Chd1* has not been identified in genetic screens for dominant modifiers of position effect variegation (PEV); we therefore did not predict that *Chd1* alleles would dominantly modify PEV. We found that loss of function of *Chd1* through RNA interference enhanced trans-allelic silencing of bw^D, while over-expression of *Chd1* resulted in an increase in gene expression. Given that *Chd1* is not an essential gene, we examined the effect of the recessive loss of *Chd1* on variegation of $In(1)w^{m4h}$ as well as lines carrying the *white* gene inserted in various locations on the fourth chromosome. When homozygous, the *Chd1* mutant allele acted as an *E(var)* of *white* inserted into the telomere of the fourth chromosome and a *Su(var)* of w^{m4h}. Thus, we observed that a loss of function *Chd1* allele can modify PEV in a manner that varies depending upon the chromosomal location of the *white*gene.

660C Chromatin remodelers, nuclear bodies and neurodegenerative diseases: from fly to humans. Luca Lo Piccolo[1,2], Rosa Bonaccorso[1,3], Andrea Attardi[1], Lorenzo Li Greci[1,4], Giorgio Giurato[5], Antonia Maria Rita Ingrassia[1], *Maria Cristina Onorati*[1]. 1) Dipartimento STEBICEF, viale delle Scienze ed.16, Universita' degli Studi di Palermo, 90128 Palermo; 2) Applied Biology Department, Kyoto Institute of Technology, Matsugasaki Sakyo.ku, Kyoto606-8585; 3) Unità di Neuroimmunologia, Ospedale San Raffaele-INSPE Via Olgettina 58 20132 Milano; 4) Turku Centre for Biotechnology, University of Turku - Biocity, Tykistökatu 6, Turku FIN-20520 Finland; 5) Genomix4Life Srl, University of Salerno, Baronissi (SA), Italy.

Over the past decade, several evidence identified a link between protein aggregation, RNA biology, and a subset of degenerative disease. Indeed, mis-regulation of RNA processing has been described in a growing number of neurological pathologies. An important feature of these disorders is the cytoplasmic or nuclear aggregation of RNA-binding proteins (RBPs). Redistribution of RBPs like the human TAR DNA-binding protein (TDP-43 or TARDBP) from the nucleus to cytoplasmic inclusions, is a pathological feature that hallmarks several diseases like sporadic and familial forms of amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration with ubiquitin-positive

inclusions (FTLD-U) and other neurodegenerative disorders including Multi System Proteinopathy (MSP). Recently, the wide spectrum of neurodegenerative diseases characterized by RBPs functions alteration and/or loss was collectively named proteinopathies. Here we show that TBPH, the *Drosophila* orthologous of human TDP-43, is a component of the nucleoplasmic omega speckles compartment, ribonucleoprotein complexes believed to function as storage sites for the unengaged hnRNPs and other related RNA-processing proteins in normal as well as stressed cells. Strikingly, loss of the omega speckles-remodeler ISWI changes dramatically TBPH localization. Indeed, TBPH disappears from the nucleus and accumulates in the cytoplasm in structures resembling the pathological inclusions seen in the tissues of patients affected by different proteinopathies. Taken together, our results show that ISWI is a leading actor in the hnRNP's nucleoplasmic compartmentalization and that its deregulation could be a key factor in the onset and development of several TBPH-related proteinopathies, open a new scenario to develop and improve new therapeutic targets and strategies against several devastating neurodegenerative proteinopathies.

661A The role of TnaA on *Hox* gene expression in third instar larvae of *Drosophila melanogaster*. *M. Rosales-Vega*, J. Murillo-Maldonado, M. Zurita, M. Vázquez. Instituto de Biotecnología. Genética del Desarrollo y Fisiología Molecular, Univ. Nacional Autónoma Mexico, Cuernavaca, Morelos, Cuernavaca, Morelos, Mexico.

Homeotic (*Hox*) genes specify the identity of body segments. Polycomb (PcG) and trithorax (trxG) groups are required for the *Hox* maintenance of transcriptional repression or activation respectively. *tonalli* (*tna*) is a trxG gene identified as a genetic modifier of *brahma* (*brm*) which encodes the ATPase of BAP and PBAP chromatin remodeling complexes in *Drosophila*. *tna* also interacts genetically with other genes from the trxG and PcG, and with genes from the transcriptional machinery. SUMOylation is a post-translational modification that can determine the binding partners of a protein leading to changes in protein stability or subcellular localization. *tna* encodes TnaA, a putative SUMO E3 ligase which favor SUMOylation target specificity.

TnaA is expressed in both, third instar larvae imaginal discs and the nervous system. Many *tna* mutant individuals die late in development from third instar larvae to pharate stages. Transheterozygote adult individuals with mutations in *tna* and in genes encoding the BAP complex show loss-of-function genotypes of the *Hox* genes *Ubx* and *Antp*, and here we hypothesize that misexpression of these *Hox* genes could be observed in haltere or wing imaginal discs or in the nervous system of *tna* mutant larvae. In this work we will show analyses of TnaA, Antp and Ubx proteins (evaluated by Westerns and larval tissue-immunostainings) in animals with altered TnaA dosages, obtained either by *tna* RNAi expression or in tna^{-} mitotic clones.

We also analyzed the regulation of *Antp* expression in wing imaginal discs. Antp has two different promoters, *P1* and *P2* that are active in different regions of the wing disc. By classical genetic analyses some years ago, we identified broadly a *P2* upstream region that is sensitive to *brm*, *osa* and *tna* dosages. We are exploring published chromatin marks or DNA accessibility data to delimit more finely this region because one possibility is that TnaA may be involved in the regulation of the chromatin landscape to determine whether this promoter is transcriptionally available.

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662B Y chromosome sequence diversity, repeat content, and phenotypic variation in the Global Diversity Lines *Emily Brown*, Keegan Kelsey, Andrew Clark. Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The Drosophila Y chromosome presents a unique context in which to study heterochromatin regulation and evolutionary dynamics. Although highly degenerated and comprised primarily of repetitive sequences, the Y chromosome nonetheless harbors natural variation with striking effects on various phenotypes, including thermotolerance, and position effect variegation (PEV). These phenotypic variations are unlikely to be due to polymorphism in protein coding genes, as the few genes on the Y chromosome show low levels of sequence variation. However, ongoing work in the Clark lab has demonstrated that there is significant variation in the repeat content of Y chromosomes in Y-replacement lines derived from the 84 Global Diversity Lines. Using assembly-naïve sequencing approaches in these lines, we have been working to both characterize variation in repeat content and abundance, and understand how sequence variation on the Y chromosome affects heterochromatin integrity, as measured by PEV.

663C Single embryo ChIP in Drosophila allows for detection of subtle changes of chromatin in embryogenesis *Carolus Chan*, Shivani Mahajan, Doris Bachtrog. Integrative Biology, UC Berkeley, Berkeley, CA.

Chromatin Immunoprecipitation Sequencing (ChIP-seq) has given much insight into the transcription factor binding sites and chromatin landscape. Specifically, using ChIP to study chromatin in Drosophila embryonic development often requires collecting embryos over the course of hours followed by formaldehyde crossing linking and sonication. This often requires large amount of starting material which makes studying subtle changes in chromatin at specific stages of Drosophila embryogenesis difficult. Here, we use an ultra low input – native ChIP to study heterochromatin

formation at specific stages of embryogenesis. Starting material can go as low as a single embryo allowing us to analysis differences in heterochromatin between sexes in embryogenesis.

664A Overexpression of HP1B and HP1C and their effects on healthspan *Tandy Dolin Petrov*, Nicole Riddle. Biology, University of Alabama at Birmingham, Birmingham, AL.

As organisms age, they often show declines in health, leading to, for example, diminished mobility and an inability to recover from stress. Associated with these age-related physiological changes are changes in chromatin structure, particularly in the heterochromatic portion of the genome. The Heterochromatin Protein 1 (HP1) family of chromosomal proteins is highly conserved in eukaryotes from yeast to humans and is important for the integrity of heterochromatin. HP1 proteins contribute to chromatin structure maintenance, transcription regulation, and DNA repair. In *Drosophila melanogaster* three somatic HP1 paralogs exist: HP1a, HP1B and HP1C. Flies that lack HP1a or HP1C do not survive to adulthood, while those lacking HP1B and all HP1 heterozygotes are viable and fertile. Interestingly, overexpressing HP1a improves health measures such as climbing ability in older flies. Currently, the effects of overexpressing the other two HP1 proteins are unknown. Our lab has generated overexpression constructs for both HP1B and HP1C. These fly lines, along with the original HP1a overexpression line, will be evaluated for impacts on chromatin structure (polytene chromosome analysis, ChIP) and health in older animals (activity levels, climbing ability, stress resistance). Results from these studies will reveal if high levels of HP1 proteins in general have a positive impact in older animals, or if this effect is unique to HP1a.

665B How heterochromatin is formed at repeats and how some genes are able to function in this restrictive environment *Sarah C R Elgin*, Jacob Cantrell, Emily Chi, Sukruth Shashikumar, April M. Bauer, Elena Gracheva. Dept Biology, Washington University, St Louis, MO.

Genomes of higher eukaryotes can be divided into two fundamental and dynamic subtypes, euchromatin and heterochromatin, which differ in patterns of histone modification and associated proteins. Heterochromatin is enriched in repetitive DNA, both tandem arrays and interspersed transposable elements (TEs); these TEs are effectively silenced by this packaging. Genes that are generally active in a euchromatic environment are silenced when transposed to heterochromatin, demonstrating Position Effect Variegation (PEV). Expansion of a triplet repeat in mammals will drive local silencing. To characterize such heterochromatin formation using *Drosophila*, we built a transgenic construct with a DNA fragment of 310 copies of the triplet GAA originating from a Friedrich's Ataxia patient, inserted upstream of an *hsp70-white* reporter. The GAA_{310}-*hsp70-white* transgene was incorporated into the genome at the base of Chr. 2L within the active euchromatic *nesd* gene but in close proximity to a heterochromatic block. At this location, *hsp70-white* yields a red-eye phenotype, while addition of GAA_{310} results in silencing of *hsp70-white*. Genetic crosses with null mutants for HP1a and SU(VAR)3-9 proteins resulted in loss of the GAA_{310}-*hsp70-white* reporter silencing, suggesting local heterochromatin formation. Preliminary genetic analyses suggest a role for the HKMT and HDAC systems in the silencing caused by GAA_{310}.

The fourth ("dot") chromosome (Muller F element) in *D. melanogaster* appears to be entirely heterochromatic, but has normal gene density and expression. The euchromatic *hsp70-white* reporter is silenced (PEV phenotype) when inserted on the 4th chromosome specifically at landing site MI03025 (Venken et al., 2008). We have used the promoter plus ~1 kb upstream region of a highly expressed 4th chromosome gene, *Rad23*, to replace the *hsp70* promoter. The newly created *Rad23-white* transgene has full expression at site MI03025 (uniform red eye phenotype) indicating that the noncoding region upstream of *Rad23* TSS is sufficient to induce full expression of the euchromatic gene in a repressive environment. Our research aims to identify heterochromatic gene regulatory elements that drive active transcription in domains targeted for silencing by repeats.

666C X-autosome translocations and the dominant disruption of male fertility *James Kennison*. Division of Developmental Biology, NICHD, NIH, Bethesda, MD.

About 77% of *X*-autosome translocations in Drosophila are male-sterile. This sterility is dominant and male-specific. The *X* chromosome breakpoints of the male-fertile *X*-autosome translocations are not random; the *X* chromosome breakpoints cluster in two regions. The non-random pattern led to the model that the *X* chromosome is precociously inactivated during spermatogenesis, and that *X*-autosome translocations disrupt this precocious *X* inactivation. There also appears to be a region in the centric *X* heterochromatin that is essential for the differential behavior of the *X* and the autosomes during spermatogenesis. One major roadblock to investigating the dominant male sterility associated with X-autosome translocations is that very few stocks with *X*-autosome translocations are currently available. We are generating a new collection of *X*-autosome translocations to test various models that could account for the dominant disruption of spermatogenesis and to map the controlling region in the *X* heterochromatin.

667A Identifying sequence interactions that underlie ring chromosome-induced embryonic lethality *Nitin Kuppanda*, Patrick M. Ferree. W. M. Keck Science Department, Claremont McKenna, Pitzer and Scripps Colleges,

Claremont, CA.

Abnormally circularized or 'ring' chromosomes derived from the normal linear sex chromosomes (X and Y) can be mitotically unstable in natural contexts and in the laboratory. Previous studies established that certain compound XY ring chromosomes produced by Oster (1964) form widespread chromosome bridges during the cleavage divisions and very high lethality. Moreover, these bridges consisted largely of 359-bp satellite DNA, which is normally located in the pericentromeric region of the normal X. Interestingly, other XY rings harboring similar amounts of 359-bp DNA were only mildly lethal to non-lethal. These observations suggested that ring lethality arises due to deleterious interactions between the 359-bp DNA and other sequences, which normally reside in different chromosomal locations but are brought together artificially in the ring context. To further explore this phenomenon, we examined a series of XY ring derivatives originating from an Xray-irradiated, highly lethal ring precursor (complements of K. Golic). We provide additional cytological and genetic evidence from these derivatives that other sequences in addition to 359-bp DNA underlie ring lethality. One candidate is the telomere-associated retrotransposon HetA, which we found to be present within and adjacent to the 359-bp DNA block in several lethal XY rings. Mutations in several Argonaut family genes, when maternally inherited, exacerbated the lethality of a moderately lethal XY ring. Finally, several replication licensing factors failed to localize to the XY ring bridges, directly demonstrating an altered chromatin state.

668B Spatial associations between genomic regions enriched with heterochromatic marks *Grace Lee*[1], Giacomo Cavalli[2], Gary Karpen[1]. 1) Lawrence Berkeley National Lab, Berkeley, CA; 2) Institute of Human Genetics, CNRS, Montpellier, France.

Pericentromeric and telomeric heterochromatin, which is enriched with repressive epigenetic marks H3K9me2/3 and heterochromatin proteins (particularly HP1a), are widely observed to coalesce and form a domain in the nucleus. This heterochromatin domain is thought to have a high concentration of proteins necessary for heterochromatin assembly and function, while depleted for proteins essential for gene expression. This spatial organization of heterochromatin can thus significantly influence gene function, which is evidenced by the silencing of euchromatic genes adjacent to translocated heterochromatin. Interestingly, in addition to being generated by large-scale chromosomal inversions and/or translocations, other types of enrichment of repressive heterochromatic marks in euchromatin are observed. This includes large genomic regions enriched with H3K9me2/3 that often contain essential euchromatic genes ("H3K9me2/3 euchromatic peaks"), as well as spreading of heterochromatic marks from epigenetically silenced transposable elements. We analyzed previously published Hi-C data to investigate if these euchromatic regions display spatial interactions with constitutive heterochromatin. We found that a significant proportion of H3K9me2/3 euchromatic peaks show 3D spatial proximity to heterochromatin. Importantly, the size of H3K9me2/3 euchromatic peaks, which likely reflects the concentration of heterochromatin proteins, are positively correlated with spatial associations, supporting the common view that enrichment of HP1a promotes the coalescence of heterochromatin. We also identified several euchromatic transposable elements, which we previously showed resulting in spreading of repressive heterochromatic marks to flanking sequences, are involved in similar 3D associations with heterochromatin. These spatial associations between euchromatic loci and constitutive heterochromatin can have significant functional consequences, such as suppression of gene expression, and can influence the evolution of genes and genomes, such as the evolution of the organization of functional elements, which we are further investigating. In addition, our analysis found that the spatial association among constitutive heterochromatin is not homogenous among different chromosomes. Heterochromatin from different arms of the same chromosome is more likely to be associated, suggesting previously unknown substructures within the seemingly homogenous heterochromatin domain.

669C An RNA Topoisomerase Complex Interacts with RNAi Machinery to Promote Heterochromatin Formation and Transcriptional Gene-silencing *S. Lee*[1], W. Shen[1], Y. Xue[1], Y. Zhang[1], M. Ahmed[1], Y. Joo[1], Y. Ding[2], S. De[1], E. Lehrman[1], K. Becker[1], K. Zhao[2], A. Sharov[1], S. Zou[1], W. Wang[1]. 1) National Institute on Aging/NIH, Baltimore, MD; 2) National Heart, Lung, and Blood Institute/NIH, Bethesda, MD.

Topoisomerases resolve topological problems generated during DNA metabolism, but the roles of topoisomerases in RNA metabolism remain unclear. Our prior study has identified human Topoisomerase 3b (Top3b) as the first RNA topoisomerase in eukaryotes, whereas another study has linked *Top3b* gene deletion to schizophrenia and intellectual disability. Mechanistically, Top3b forms a stoichiometric complex with TDRD3 (Tudor domain containing 3); and a fraction of this complex associates with FMRP, an RNA-binding protein known to regulate translation of mRNAs important for synapse development and autism. FMRP is encoded by the *fmr1* gene, which is inappropriately silenced in the Fragile X mental retardation syndrome, a leading cause of autism. Using *Drosophila* as a model, we showed that *Top3b* genetically interacts with *fmr1* to promote synapse formation.

Increasing evidence has shown that *Drosophila* FMRP is a component of the RNAi-induced silencing complex (RISC), which includes AGO2, p68 helicase, and Vig. Mutations in *fmr1*, other components of RISC, as well as *Dicer-2* (*Dcr-2*; a protein essential for siRNA biogenesis), disrupt heterochromatin formation, transcriptional gene silencing, and repression of transposable elements. Here, we show that similar to FMRP, the *Drosophila* Top3b-TDRD3

complex also stably associates with RISC; and mutation of *Top3b* disrupts heterochromatin formation and transcriptional silencing in Position Effect Variegation (PEV) reporter assays. In addition, *Top3b* genetically interacts with *AGO2, p68,* and *Dcr-2* in PEV assays, indicating that Top3b works coordinately with the siRNA machinery to facilitate heterochromatic gene silencing. Our CHIP-sequencing analysis demonstrates that heterochromatin epigenetic markers, Heterochromatin Protein 1 (HP1), H3K9me3, and H3K9me2, display abnormal distribution at pericentric and telomeric chromosomal regions in *Top3b* mutant flies. Furthermore, microarray and RT-qPCR assays show increased gene expression at the above telomeric and pericentric heterochromatin regions. Together, our data suggest that the RNA topoisomerase Top3b interacts with the siRNA machinery to promote heterochromatin formation and transcriptional silencing.

670A Heterochromatin-induced Gene Silencing is Decided Early and Set Late. *Keith Maggert*. Cellular and Molecular Medicine, University of Arizona, Tucson, AZ.

Genes inappropriately moved into or out of heterochromatic regions of the genome exhibit stochastic and mitotically-heritable expression states. These patterns of Position Effect Variegation (PEV) have been a foundation of "epigenetic" inheritance phenomena, whose behaviors are inextricably linked to heterochromatin biology. The patterns of PEV vary based on the heterochromatic locus in question, although a clear molecular explanation for the different patterns remains unknown. Different patterns of PEV are thought to reveal differences in the developmental timing of the onset of heterochromatin-induced silencing, although it is also possible that patterns arise from infidelities of silencing as cells pass through the cell cycle.

Using analysis of patterns of PEV in adult tissue, I argue that the fidelity of silencing is programmed very early in development (prior to, or at, fertilization), and decay independently in individual developmental cell lineages. To explore silencing in live tissues, I developed a novel multicomponent reporter system (SwiM, for Switch Monitoring) to independently monitor gains and losses of heterochromatin-induced silencing during development, and to monitor its stability in live post-mitotic cells. Using the SwiM system, I observed cells losing and gaining silencing through development, indicating that heterochromatin-induced silencing is not set at specific times in development, but rather is dynamic until differentiation. Drugs and mutations that disrupt silencing do so at different times in development, indicating they act on different aspects of heterochromatin formation and function. Lineages of cells vary in their potential to be silenced by heterochromatin, accounting for the final adult patterns of PEV. Adapting the SwiM System to capture the contents of cells undergoing switching of silencing, I detect genome changes in rDNA copy number, a lesion known to quantitatively affect heterochromatin formation. This counter-intuitive finding argues that heritable differences in heterochromatin-induced gene silencing may arise from accumulated somatic genetic differences in cell lineages, challenging core notions of the canonical "epigenetic" establishment and maintenance of inherited gene expression states.

671B Heterochromatin formation in *D. miranda* during embryonic development *Shivani Mahajan*. Integrative Biology, UC Berkeley, Berkeley, CA.

Heterochromatin - the tightly packed repeat-rich and gene-poor chromatin compartment - is established during early development, however, little is known about how heterochromatin formation is initiated. Sex chromosomes evolve from a pair of ordinary autosomes and over the course of its evolution the Y chromosome degenerates, that is, it looses its ancestral genes and instead accumulates repetitive sequences. This is accompanied by a transition of its chromatin structure from a euchromatic autosome to a heterochromatic Y chromosome. In *D. melanogaster*, the ancestral Y chromosome is entirely heterochromatic and poorly assembled, and is thus too old to study the causes of changes in chromatin structure. *D. miranda* has a pair of newly evolved sex chromosomes (neo-sex chromosomes) that formed approximately 1MY ago which are at an intermediate stage of their transition from a pair of ordinary autosomes into a pair of heteromorphic sex chromosomes. The neo-Y still contains 1000s of unique genes, but has begun to accumulate repetitive DNA and evolve a heterochromatic chromatin conformation. Here, we use ChIP-seq to identify regions targeted by repressive marks (such as H3K9me2/H3K9me3) as well as activating marks (such as H4K16ac and H3K4me3) throughout the maternal-to-zygotic transition in embryos of *D. miranda*, in order to characterize heterochromatin formation during embryonic development in Drosophila.

672C Testing the heterochromatin sink hypothesis through genome size variation *Alison Nguyen*, Doris Bachtrog. University of California, Berkeley, Berkeley, CA.

Previous studies have shown that the Y chromosome, which is highly repetitive and enriched for heterochromatin, can alter the epigenetic landscape and influence the expression of thousands of genes. It is believed that the D. melanogaster Y chromosome acts as a “heterochromatin sink” such that its repetitive sequences accumulate components required for heterochromatin formation and deplete these epigenetic components elsewhere. Under the “heterochromatin sink” model, males with larger Y chromosomes should sequester more repressive marks on their Y compared to males with shorter Y’s. To test if Y chromosome size variation can change the genomic distribution of heterochromatin, we established lines whose males carry unique Y chromosomes on isogenic backgrounds (Y-

replacement lines). We used flow cytometry to characterize size variation among Y chromosomes and position effect variegation assays and ChIP-Seq for heterochromatin-specific histone modifications to identify changes in the epigenetic landscape. Our data will resolve whether repetitive content on the Y influences the stoichiometric balance of histone modifications genome-wide and RNA-seq data will allow us to evaluate its effect on overall genome-wide expression.

673A Elba and Insensitive are involved with GAF into Fab-7 boundary activity *Francois Karch*[1], Bastien Desplands[1], Mikaël Rossier[1], Annick Mutero[1], Robert K. Maeda[1], Rakesh Mishra[2]. 1) Dept Genetic and Evolution, Univ Geneva, Geneva, Switzerland; 2) Centre for Molecular and Cellular Biology, Hyderabad, India.

The sequential activation of the bithorax complex segment-specific functions occurs through the progressive opening of cis-regulatory domains (Bowman et al., 2014). Critical to this mechanism are the boundary elements that delimit the expanse of each regulatory domain. For example the *Fab-7* boundary delimits the border between the *iab-6* and *iab-7* regulatory domains. In the absence of *Fab-7*, *iab-6* active status in A6 spreads into *iab-7* resulting into a GOF homeotic mutation in which A6 is transformed into A7. At the molecular level, *Fab-7* is characterized by the presence of 3 prominent nuclease hyper-sensitive regions, HS1, HS2 and HS3. HS3 corresponds to a Polycomb-Response-Element (the so-called *iab-7*PRE). Using a phiC31 based *Fab-7* integration platform we recently showed that the elements necessary for *Fab-7* lie within HS1 and depends on the integrity of 6 GAGAG binding sequences for GAF (Wolle et al., 2015). During the course of these studies, we had also created a *Fab-7* boundary in which 5 of the 6 GAGAG motifs were mutated. This chromosome is associated with a weak *Fab-7* phenotype of very low penetrance. It turned out to be very useful to characterize the Elba/Insv factors identified by Aoki et al (2012; and also by us in a completely different strategy) to bind at 2 sites within HS1. While mutating these binding sites alone has no consequence on *Fab-7* boundary, adding these 2 mutations on top of the 5 GAGAG site mutations greatly enhance the strength and the penetrance of the *Fab-7* phenotype. Furthermore, using a CRISPR/Cas9 strategy we were able to recover mutations in Insv and Elba2 on the basis of their enhancement of the Fab-7(GAGAG1-5) phenotype. These results suggest that the Elba/Insv proteins act as co-factor of GAF into *Fab-7* boundary function.

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674B ATR and ATM Modulate Chromatin Insulator Activity and Genome Stability Through Phosphorylation of Histone H2Av at Insulator Sites Ran An, Shannon Garland, Shih-jui Hsu, *Mariano Labrador*. Biochem Cell & Molec Biol, Univ Tennessee, Knoxville, TN.

Traditionally, chromatin insulators are considered to function as chromatin boundaries and enhancer blockers, because of their ability to prevent the spreading of heterochromatin in the chromatin fiber and to block enhancer-promoter interactions when located between an enhancer and a promoter. A broader interpretation of the role of insulators is to maintain the stability of the tridimensional organization of the genome, given that they can promote the formation of higher order chromatin structures by stabilizing interactions between distant genomic sites along the chromatin fiber. Because of these properties, insulators contribute to gene transcription regulation by selectively allowing looping contacts between gene regulatory regions and promoters. However, the mechanism of insulator function, or the specific role that insulators play in genome architecture and how this architecture is maintained during cell division, differentiation and different stress responses, remains unclear. Here, we find that the *Drosophila* Suppressor of Hairy wing insulator protein [Su(Hw)] co-localizes with the phosphorylated form of the histone variant H2Av (γH2Av) in chromosomes. γH2Av is a marker for double strand breaks (DSB), and functions in the DNA damage repair pathways regulated by ATR and ATM, two kinases that phosphorylate H2Ax in humans and the equivalent H2Av in *Drosophila*. We found that loss of the enhancer-blocking function of the *Gypsy* insulator correlates with loss of γH2Av, which partially restores the phenotype of *Gypsy* induced mutations to wild type. These results suggest that phosphorylation of H2Av at insulator sites is required for proper insulator function. Supporting this notion, we found that loss of function of the *Drosophila* ATM and ATR (*tefu and mei-41* respectively) affect the expression of insulator proteins and also produces different degrees of phenotypic suppression on the y^2 and ct^6 alleles. Mutations in *me1-41* also partially rescue the sterility phenotype in ovaries of *Su(Hw)* mutant females. In addition, we show γH2Av associates with insulator bodies during osmotic stress, and that the level of phosphorylation of H2Av is regulated by Protein Phosphatase PP2A such that insulator bodies are more stable with higher levels of H2Av phosphorylation. These results Indicate that ATR and ATM are required for insulator function by mediating phosphorylation of H2Av and suggest a novel mechanism that links genome organization and insulator function with the mechanisms that control genome homeostasis and stability.

675C *Drosophila O-GlcNAcase* Deletion Globally Perturbs Chromatin *O*-GlcNAcylation *Ilhan Akan*[1], Dona Love[2], John Hanover[1]. 1) LCMB / NIDDK, National Institutes of Health , BETHESDA, MD. 20892; 2) National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA, 20892.

Gene expression during *Drosophila* development is subject to regulation by the Polycomb (Pc), Trithorax (Trx) and Compass chromatin modifier complexes. *O*-GlcNAc Transferase (OGT/SXC) is essential for Pc repression suggesting that the *O*-GlcNAcylation of proteins plays a key role in regulating development. OGT transfers *O*-GlcNAc onto serine and threonine residues in intrinsically disordered domains of key transcriptional regulators; *O*-GlcNAcase (OGA) removes the modification. To pinpoint genomic regions that are regulated by *O*-GlcNAc levels, we performed ChIP-chip and microarray analysis after OGT or OGA RNAi knockdown in S2 cells. After OGA RNAi, we observed a genome-wide increase in the intensity of most *O*-GlcNAc-occupied regions including genes linked to cell cycle, ubiquitin and steroid response. In contrast, *O*-GlcNAc levels were strikingly insensitive to OGA RNAi at sites of polycomb repression such as the Hox and NK homeobox gene clusters. Microarray analysis suggested that altered *O*-GlcNAc cycling perturbed the expression of genes associated with morphogenesis and cell cycle regulation. We then produced a viable null allele of *oga* ($oga^{del.1}$) in *Drosophila* allowing visualization of altered *O*-GlcNAc cycling on polytene chromosomes. We found that Trithorax (TRX), Absent small or homeotic discs 1 (ASH1) and Compass member SET1 histone methyl-transferases were *O*-GlcNAc-modified in $oga^{del.1}$ mutants. The $oga^{del.1}$ mutants displayed altered expression of a distinct set of cell cycle related genes. Our results show that the loss of OGA in *Drosophila* globally impacts the epigenetic machinery allowing *O*-GlcNAc accumulation on RNA Polymerase II and numerous chromatin factors including TRX, ASH1 and SET1

676A De novo recruitment of PcG proteins at *giant* *Jumana AlHaj Abed*[1], Elnaz Ghotbi-Ravandi[2], Piao Ye[2], Alex Frolov[2], Judith Benes[2], Rick Jones[2]. 1) Genetics, Harvard Medical School, Boston, MA; 2) Biological Sciences, Southern Methodist University, Dallas, TX.

Polycomb group proteins (PcG) are a highly conserved epigenetic regulators that maintain the transcriptional repression of their target genes. They do not initiate transcriptional repression of target genes but rather take over repression from gene-specific transcription factors. PcG-mediated repression can be defined into two stages, establishment of PcG transcriptional repression, followed by heritable maintenance of the silenced state through an indefinite number of cell divisions. Studies of *Drosophila* embryos have shown that PcG proteins do not initiate transcriptional repression, but rather take over repression from gene-specific transcription factors. Once PcG-mediated repression is established, it is stably maintained through numerous cell cycles. Very little is known about the molecular mechanisms by which PcG proteins initially recognize the repressed state of a target gene, and take over its repression. This is due to the technical limitations imposed by heterogeneous expression of target genes within developing embryos during that time.The goal of this study is to better define the molecular and biochemical steps involved in the initial recognition of a repressed target gene by PcG proteins and the establishment of PcG-mediated repression. For this purpose, we focus on the PcG target gene *giant* (*gt*) in embryos from *bcd osk tsl* females. In these embryos, after the initial ubiquitous repression of *gt* by maternally expressed Hunchback at syncytial blastoderm, PcG is required to maintain its ubiquitous repression by cellular blastoderm stage. We have performed time course chromatin immunoprecipitiation (ChIP) experiments using carefully staged embryos to examine the distributions of PcG proteins as well as a repressor, Hb, and an activator, Cad, at *gt* during the developmental window at which repression transitions from Hb to repression by the PcG proteins. Our studies reveal clear localization of Pho at *gt* PREs at a very early stage of development. In addition, we show a subtle but clear correlation between recruitment of Enhancer of zeste [E(z), component of PRC2] and an increase of H3K27me3 across the *gt* locus. This is immediately followed by a clear and stable increase in the localization of Polycomb (Pc, a component of PRC1). These observations support a model for PcG recruitment in which PcG complexes initially are loosely associated with target genes before becoming more stably associated and forming a repressive chromatin environment. The timing of these events also support a role for H3K27me3 in stable recruitment of PRC1.

677B The recognition of target gene transcriptional state by Polycomb Group proteins. *Elnaz Ghotbi Ravandi*, Piao Ye, Jumana AlHaj Abed, Judith Benes, Richard Jones. Dept of Biological Sciences, Southern Methodist University, Dallas, TX.

Polycomb Group (PcG) proteins are evolutionarily conserved epigenetic transcriptional regulators that maintain the transcriptional repression of silenced genes by altering chromatin structure. After initial recognition and binding of PcG proteins to their repressed target genes, they are able to maintain the transcriptional repression through an unlimited number of cell cycles. Most studies on PcG proteins have been focused on the maintenance phase of PcG silencing, thus the molecular mechanisms by which PcG proteins initially recognize a repressed gene and the establishment of PcG-mediated repression remain unknown. This is mainly due to the technical limitations imposed by heterogeneous expression of target genes within developing embryos during the time. By collecting embryos from *bcd osk tsl* females, we are able to obtain a population of nuclei in which *giant* (*gt*), a PcG-target gene, is uniformly repressed by PcG proteins. Time course ChIP experiments using embryos at multiple distinct embryonic

stages indicate the weak presence of PcG proteins at *gt* in syncytial blastoderm stage prior to their stable binding at the end of cellular blastoderm. Ongoing work is designed to determine whether binding by particular transcription factors or the transcriptional state of a target gene identifies PcG target loci as initially repressed. In addition, we are examining the roles of individual PcG proteins and other transcription factors in recruitment of PcG complexes and initial establishment of PcG-mediated repression. Progress on these studies will be presented.

678C Epigenetics Factors Involved in Feedback Regulation of Hox Homeostasis *Devendran Sadasivam*, Der-Hwa Huang. Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

Pluripotent stem cells often adopt a unique developmental program while retaining certain flexibility. Hox genes are critical for the selection of specific developmental direction of pluripotent tissues. Therefore, the transcriptional regulation of Hox genes controlled by two groups of epigenetic factors called Polycomb group (PcG) of repressors and Trithorax group (trxG) of activators plays an important role in maintenance of pluripotency. Although these factors often co-exist in the same cells, it is poorly understood how the homeostatic equilibrium is maintained between them. Previously, we showed that Trx over-expression, a H3K4 methyltransferase, can induce ectopic adult appendages by selectively activating Hox gene in specific imaginal discs (Sadasivam and Huang, 2016). The tissue-specific inducibility correlates with the presence of H3K4me3 and paused RNA polymerase II in promoter-proximal region of these genes. Lineage tracing and pulse-chase experiments revealed that the active state of Hox genes is maintained substantially longer in mutants deficient for HIRA, a chaperone for the H3.3 variant. In addition, both HIRA and H3.3 appeared to act cooperatively with the PcG. Recently, we found that Trx over-expression results in transient induction of PcG, thereby preventing continuous ectopic Hox expression. Thus, in addition to the activation of specific Hox promoters through multiple epigenetic factors, Trx may trigger a feedback regulatory loop to maintain homeostatic equilibrium.

679A Investigating the role of nuclear pore proteins in regulating chromatin structure and gene expression *Terra Kuhn*, Maya Capelson. Cell and Developmental Biology Department, University of Pennsylvania, Philadelphia, PA.

Nuclear pore complexes (NPCs) are large protein complexes spanning the eukaryotic nuclear membrane. They are comprised of multiple copies of ~30 proteins called nucleoporins (Nups) and regulate mRNA and protein transport between the nucleus and cytoplasm. In recent years, studies have demonstrated that these complexes play a role in regulating chromatin state and gene expression. Studies show that interaction of specific recently activated genes with NPCs in yeast or with "off-pore" intranuclear Nups in metazoan cells induces enhanced expression and long-term transcriptional memory of target loci. Additionally, interaction of target genes with Nups is associated with changes in histone modifications and composition of nucleosomes, as well as nucleosome positioning. Critically, many target loci are developmentally important genes, such as Hox genes, with expression dependent upon Nup binding. It is clear that NPCs and their component proteins play an important role in regulating chromatin state and gene expression, but how these proteins conduct this regulation is not well characterized. In order to study the chromatin and gene expression related functions of individual Nups, we have generated a system ectopically tethering Nups to specific loci in the *Drosophila* genome by expressing LacI-Nup fusion proteins in flies containing an integrated *lacO* repeat array. Using this system, we are able to test chromatin structure changes and recruitment of chromatin and transcription related proteins by specific Nups *de novo,* using immunofluorescence in polytene chromosomes from larval salivary glands. In addition, this system allows us to assess changes in transcription of reporter genes downstream from the *lacO* sequence. Interestingly we have identified a unique role of Nup98 and Sec13 to decondense chromatin at target *lacO* loci, a function not shared by fellow Nups. This is consistent with recent findings from our lab showing an interaction and functional relationship between Nup98 and active chromatin modifiers MBD-R2 and Trx. We are currently investigating whether Nup98 and other Nups are sufficient to recruit key histone-modifying and chromatin remodeling complexes to the target ectopic loci, in order to gain a mechanistic understanding of how Nups regulate chromatin structure and gene expression in animal cells.

680B Dosage compensation complex as a target of male killing *Spiroplasma* in *Drosophila melanogaster* *Becky Cheng*, Patrick M. Ferree. W. M. Keck Science Department, Claremont McKenna, Pitzer and Scripps Colleges, Claremont, CA.

Drosophila melanogaster and other insects harbor intracellular bacteria that are transmitted vertically from infected host mothers to their offspring. Many of these bacteria alter host reproductive processes in order to increase bacterial transmission success. *Spiroplasma*, a spirochete that naturally infects *D. melanogaster*, selectively kills males during mid embryogenesis while sparing females. Previous studies suggested that *Spiroplasma* genetically interacts with the male-specific dosage compensation pathway, which upregulates expression of genes located on the male's single X so that their expression levels match those found in females, which have two Xs. To further investigate this interaction, we transgenically expressed MSL2 in both *Spiroplasma*-infected and uninfected female embryos in order to cause ectopic formation of the dosage compensation complex (DCC). Infected female embryos expressing the

DCC showed significantly reduced viability compared to uninfected DCC+ females. This result supports the notion that *Spiroplasma* uses the DCC in a dominant gain-of-function manner to kill embryos. We also used confocal microscopy to visualize DCC localization and activity in infected and uninfected embryos. In the presence of *Spiroplasma*, the DCC became abnormally localized across the nucleus. This pattern accompanied abnormal acetylation of histone H4K16, a mark induced by DCC activity that is needed for proper X chromatin remodeling. Our results imply that *Spiroplasma* directly alters the DCC in a way that misdirects it to uncompensated regions of the genome, an effect that leads to abnormal gene mis-regulation (work from other lab members) and consequent lethality.

681C Small RNAs and the epigenetics of X-recognition *Nikita Deshpande*, Victoria Meller. Dept. of Biological Sciences, Wayne State University, Detroit, MI.

Eukaryotic genomes are organized into large domains of coordinated regulation. The role of small RNAs in formation of these domains is largely unexplored. An extraordinary example of domain-wide regulation is X chromosome compensation in *Drosophila melanogaster* males. This process occurs by hypertranscription of genes on the single male X chromosome. Extensive research in this field has shown that the Male Specific Lethal (MSL) complex binds X-linked genes and modifies chromatin to increase expression. The components of this complex, and their actions on chromatin, are well studied. In contrast, the mechanism that results in exclusive recruitment to the X chromosome is not understood. Prior studies in the lab have found that the siRNAs produced from repetitive sequences on the X chromosome and the repeat DNA itself, participates 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 siRNA influences X-recognition by an indirect and novel mechanism. For example, Ago2-containing complexes could bind nascent RNAs from the X chromosome and recruit activities that alter chromatin structure. An X-specific chromosome conformation might facilitate MSL recruitment and spreading into X chromatin. To test this model, I performed a genetic screen that discovered numerous *Ago2* interactors, including *Su(var)3-9*, that participate in dosage compensation. I hypothesized that repeats on the X were enriched in H3K9me2 through a siRNA-dependent mechanism. I tested this by Chromatin Immunoprecipitation (ChIP), finding that X-linked repeat sequences are in fact enriched for H3K9me2, and that this mark depends on Su(var)3-9, but not SetDB1 or G9a, the other enzymes that deposit the H3K9me2 mark. I have also demonstrated that genetic manipulations of the siRNA pathway disrupt H3K9me2 at and around repeats on the X chromosome. I am now using ChIP-qPCR to determine whether Ago2 or Su(var)3-9 themselves localize at the repeats. My research aims to determine how simple repeats can have such a profound effect on chromosome behavior and gene expression. As repeats make up a large proportion of eukaryotic genomes, it is possible that similar regulatory pathways are widespread.

682A *roX1* and *roX2* in *Drosophila melanogaster* are redundant but have distinct functions in dosage compensation *Maria Kim*, Marie-Line Faucillion, Jan Larsson. Department of Molecular Biology, Umeå University, Umeå, Sweden.

Many eukaryotes have acquired sex chromosomes independently in evolution and therefore faced the problem of gene dose imbalance. The complex mechanisms maintaining equal expression from unequal number of sex chromosomes are termed “dosage compensation”. In both mammals and *Drosophila,* males are the heterogametic sex, carrying two diverged sex chromosomes, but the strategies of dosage compensation adopted by these taxonomic groups are different. In mammals, one of the female X chromosomes is almost entirely silenced, while the transcriptional output from the only active X chromosome is increased in both sexes. In *Drosophila*, the male X chromosome is upregulated as a result of at least one histone modification and likely by other less described mechanisms. Despite the difference in mammalian and *Drosophila* dosage compensation strategies, they both depend on lncRNAs. The *Drosophila* male-specific lethal complex contains two lncRNAs, *roX1* and *roX2* which prevent the complex from mistargeting to silenced chromatin. These lncRNAs are different in size, share very little sequence homology and are transcribed at different level. Nevertheless, they are essential but redundant for male viability. We decided to address in detail the functional redundancy of *roX1* and *roX2* RNAs. A variety of *roX1* mutant alleles are available, but all previous studies of *roX2* mutants were carried out on flies with a genomic deficiency overlapping *roX2* and three adjacent genes. We have generated an almost precise deletion of *roX2* gene using the CRISP-Cas9 tool. Indeed, both single *roX* mutants are viable and fertile. We have performed next-generation sequencing of *roX1*, *roX2* and double-mutant first instar larvae transcriptomes. Here we present the detailed analysis of X chromosome dosage compensation carried out by MSL complex containing either of the two *roX* RNAs. We also describe the transcriptional responses in single and double *roX* mutants. In addition we show that the expression of the two lncRNAs is differentially regulated during the cell cycle.

683B A luciferase reporter for recruitment of dosage compensation in *Drosophila melanogaster* *Reem Makki*, V. H. Meller. Biological Sciences, Wayne State University, Detroit, MI.

Drosophila melanogaster males have one X chromosome while females have two. This creates a potential imbalance in X to autosome gene expression between the sexes. To maintain an appropriate expression ratio, male fruit flies increase transcription from X-linked genes. This is accomplished by the Male Specific Lethal (MSL) complex, which binds transcribed X-linked genes and modifies chromatin to increase expression. The complex is thought to assemble at Chromatin Entry Sites (CES), which contain the MSL recognition Element (MRE), and then spread in *cis* to active genes in the vicinity. Since the MRE is present on autosomes it is unclear how the MSL complex recognizes X-chromatin. We found that repetitive sequences that are near-exclusive to the X chromosome, the 1.688^x satellite repeats, promote recruitment of the MSL complex to nearby genes. We have developed a dual-luciferase reporter assay to measure the ability of DNA sequences to recruit compensation. *Firefly* luciferase was placed on a transgene containing the 1.688^X repeats and *Renilla* luciferase was placed on a transgene devoid of recruiting elements. Autosomal insertions of these reporters will be used to screen RNAi lines to identify genes necessary for recruitment of compensation by 1.688^X repeats. This assay will allow us to differentiate the recruiting pathways used by different types of DNA sequences. As 1.688^X sequences appear to recruit by a different mechanism than the CES, we expect to identify genes not previously known to participate in X recognition.

684C Developing hybrid cell lines as a model for understanding homolog pairing *Jumana AlHaj Abed*[1], Jelena Erceg[1], Sonny Nguyen[1], Anton Goloborodko[2], Leonid A. Mirny[2,3], Ting (C.-ting) Wu[1]. 1) Genetics, Harvard Medical School, Boston, MA; 2) Department of Physics, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA; 3) Institute for Medical Engineering and Science, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA.

Homolog pairing facilitates a variety of biologically important processes ranging from the initiation of X-inactivation and double-strand-break repair to gene regulation. It occurs in *Drosophila* somatic cells, starting in early embryogenesis and persisting throughout adulthood. Studies have shown it perdures throughout the cell cycle, albeit at varying degrees, with the G1 stage showing a maximum level of pairing at approximately 75-80%. Due to the persistence of homolog pairing it supports *Drosophila* as an ideal model system for studying *trans-* interactions.

This study characterizes hybrid cell lines produced from crosses of highly divergent *Drosophila* parents. Metaphase spreads demonstrate the lines to be diploid, and fluorescence in situ hybridization (FISH) with homolog specific Oligopaints (HOPs) confirm that the lines are indeed hybrid lines with respect to the second and third chromosomes. We report ~10 male hybrid diploid lines from one hybrid cross, and at least one male and one female hybrid line from the reciprocal cross.

This is part of a larger project using Hi-C to uncover *trans*- homolog contacts in early embryo development using stringent SNP-based filtering to distinguish paternal-maternal interactions. The same analysis pipeline will be applied to the hybrid lines from reciprocal crosses and used to produce Hi-C maps for *trans*- homolog contacts. The lines will be further characterized with FISH in order to validate regions of high contact probability and then compare such regions to those with lower contact probability. Additional work will correlate the *trans-* contact data with RNA expression and provide a complementary approach to perhaps better understand how homolog pairing and gene expression are related. This work was supported by awards from NIH (LAM, C-tW), Harvard Medical School (C-tW,) and EMBO (JE).

685A A DNA-FISH Investigation of the Relationship between Homologous Chromosome Pairing and Transvection in *Drosophila* *Thomas King*, Justine Johnson, Jack Bateman. Department of Biology, Bowdoin College, Brunswick, ME.

Transvection is a phenomenon that occurs when enhancer sequences on one chromosome interact in *trans* with promoters on neighboring chromosomes, leading to alternative gene expression patterns. When the same reporter transgene construct is inserted at random loci in the *Drosophila* genome, the amount of transvection observed varies depending on the genomic position of the construct. While chromatin structure and other epigenetic factors are likely involved in this phenomenon, chromosome pairing provides a potential alternative explanation. In the interphase nucleus in *Drosophila*, homologous chromosomes are paired (closely associated) along their entire length. If significant differences in pairing between different loci exist throughout the *Drosophila* genome and are conserved across all cells of a given cell line or tissue, these differences could influence the amount of transvection observed at a given locus. Using DNA-FISH with libraries of oligopaint probes targeting five euchromatic loci, I visualized the degree of pairing in three cell types: S2R+, BG3.13, and eye discs. I then used transvection data obtained from qualitative reporter gene levels and qPCR to investigate the relationship between pairing and transvection at the loci of interest. I found that pairing levels differed significantly across cell lines, and varied across the genome within individual cell lines. However, I found no evidence for a positive correlation between chromosome pairing and the strength of transvection. These results imply that alternative epigenetic factors, rather than pairing, are the main determinants of the strength of transvection.

686B Combined Ectopic Expression of Myb and Mip120 Causes Tumors in *Drosophila* Larvae *P.J. Vorster*, J.S. Lipsick. Stanford University, Stanford, CA.

During development genetically homogenous cells express genes differentially by epigenetic mechanisms. The *Drosophila* RBF E2F2 and Myb-interacting proteins (dREAM) complex plays a vital role in the epigenetic regulation of gene expression in dividing cells. RBF, E2F2 and DP are repressors, while the Myb proto-oncogene is the main activator of the complex. There are three copies of Myb in humans, A-MYB, B-MYB and C-MYB, all of which have been implicated in human cancer. C-MYB is recurrently translocated in acute lymphocytic leukemia, adenoid cystic carcinoma and is often duplicated or overexpressed in pediatric brain cancer. B-MYB expression correlates with poor prognosis in human breast cancer. The Myb-interacting protein of 120 kDa (Mip120) is part of the multiprotein dREAM complex. A role for Mip120 in tumorigenesis remains undefined.

Oncogenesis is a multistep process that results from mutations in oncogenes and tumor suppressors. To accurately simulate the clonal nature of tumorigenesis as seen in human cancers, a system is needed where mutant cells over proliferate within a wild-type cellular environment. *Drosophila melanogaster* provides us with the tools to overexpress and knock-down genes tissue specifically, within an otherwise wild-type background. Propagation of tumorigenesis in *Drosophila* by single mutation alone is often not possible, as single clones fail to proliferate in the presence of wild-type cells. Conversely, overexpression of single genes that cause proliferation, like activated Ras, induces hyperplasia, but not metastasis. Formation of hyperplasia and metastasis is therefore often studied in a system where single cells are manipulated to simultaneously over proliferate and lose planar cell polarity.

In an attempt to study epigenetic regulation by the dREAM complex during *Drosophila* eye development, we established a new system for propagating tumorigenesis in flies. While Myb functions as an oncogene in vertebrates, expression of Myb or Mip120 alone did not cause any detected hyperplasia in the *Drosophila* developing eye. We noticed a stark difference when we overexpressed both Myb and Mip120 simultaneously. Combinatorial expression of Myb and Mip120 caused solid tumors that formed rapidly in 3rd instar larvae. Our newfound system allows us to study the role of the dREAM complex in tumorigenesis. In particular we will focus on the molecular mechanisms involved in transcription regulation by the dREAM complex. Our model will also be useful to elucidate the role of Mip120 as a novel oncogene

687C Chromatin remodelers involved in HP1a telomeric localization *Viviana Valadez*[1], J. Chavez[1], J.M. Murillo-Maldonado[1], V. Bahena[1], A. Castañeda-Sortibran[2], R. Rodriguez-Arnaiz[2], M. Zurita[1]. 1) Genetica del Desarrollo, Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; 2) Laboratorio de Genetica. Facultad de Ciencias. UNAM, Ciudad de México, México.

Telomeres are important contributors to genome stability, they prevent linear chromosome end degradation and contribute to avoid telomeric fusions. An important component of the telomeres is the Heterochromatin Protein 1a (HP1a). Mutations in *Su(var)205,* the gene encoding HP1a, result in telomeric fusions, retrotransposon regulation loss and larger telomeres leading to chromosome instability. In this work using immunofluorescence techniques, we analysed the localization of HP1a protein in mutant backgrounds of two previously described chromatin remodelers. We found that these proteins contribute to the maintenance of chromosomal stability in part by maintaining HP1a telomeric localization.

688A Condensin II subunit Cap-H2 is required for maintenance of telomere stability *Heather A. Wallace*[1,2], Vibhuti Rana[3], Huy Q. Nguyen[2], Giovanni Bosco[3]. 1) Division of Genetics, Brigham and Women's Hospital, Boston, MA 02115; 2) Department of Genetics, Harvard Medical School, Boston, MA 02115; 3) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth.

Telomeres are nucleoprotein complexes located at the ends of eukaryotic chromosomes that contribute to genome stability by preventing inappropriate recognition of chromosome ends as double strand breaks and by protecting them from incomplete replication. Drosophila telomeres differ from those of other eukaryotes in that the core proteins forming the protective cap are not conserved and telomere length is maintained by a telomerase-independent mechanism involving targeted transposition of *HeT-A*, *TART*, and *TAHRE* retrotransposons. Despite these differences, the Drosophila telomere capping complex, terminin, is thought to be functionally analogous to the human shelterin complex. Furthermore, a number of proteins required for telomere maintenance, including HP1 homologs and ATM/ATR kinase, have conserved telomeric functions. We have identified a novel role for the Drosophila condensin II component Cap-H2 and its human ortholog NCAPH2 at Drosophila and human telomeres, respectively. Condensins are conserved multi-subunit protein complexes that contribute to chromosome organization by regulating chromosome compaction and homolog pairing. We have shown that condensin II components co-localize with terminin proteins HOAP and HP1 on telomeres of polytene chromosomes. We show that HOAP and Hiphop localization to telomeres requires Cap-H2 and loss of Cap-H2 results in formation of telomere association. Cap-H2 is necessary to maintain telomere length by positively regulating expression of the telomeric *HeT-A* retrotransposon. In human cells, we show that NCAPH2 binds to telomeric chromatin and interacts with shelterin component TRF1. Depletion of NCAPH2 results in ATR-dependent accumulation of telomeric DNA damage markers and an increase in

telomeric RPA foci, suggesting a role for NCAPH2 in telomere replication. These observations support the possibility that condensin complexes might have a conserved role in eukaryotic telomere maintenance.

689B The histone demethylase KDM5 as a potential new anti-apototic factor. *Coralie Drelon*, Xingyin Liu, Julie Secombe. Genetics, Albert Einstein College of Medicine, Bronx, NY.

KDM5 proteins are highly conserved histone demethylases that can activate or repress gene expression in a context-dependent manner. In addition to their well-described JmjC catalytic domain, KDM5 proteins also contain a JmjN domain, an ARID DNA binding domain, a C_5HC_2 zinc finger of unknown function and two or three histone binding PHD motifs. Whereas mammalian cells encode four KDM5 paralogs KDM5A, KDM5B, KDM5C and KDM5D, *Drosophila* has a single KDM5 ortholog, making of flies a good model to study the biology of KDM5 family proteins. Because knockout of mouse KDM5B or *Drosophila kdm5* results in lethality, KDM5 proteins play essential roles in transcriptional regulation during development. The dysregulation of KDM5 proteins also leads to disease, as overexpression of KDM5A or KDM5B are observed in prostate, breast and gastric cancers. However the roles of KDM5 proteins in physiological contexts and in tumor development remain poorly understood. To study the biology of KDM5, our lab carried out RNA sequencing experiments of *kdm5* mutant animals. These analyses highlighted alterations in genes required for apoptosis that are the focus of this project. To define the role of KDM5 in programmed cell death, we first tested whether KDM5 was required for developmentally regulated cell death events (e.g. pupal retina). Based on our observation that this type of cell death was unaffected in *kdm5* mutants, we tested whether KDM5 was required to respond to cellular insults such as irradiation. To do this, *kdm5* null mutant and wild type larvae were irradiated and apoptosis was detected by immunostaining for cleaved caspase Dcp1, highlighting an increase for mutant larvae. Consistent with these data, knocking down KDM5 using engrailed-Gal4 driver increased programmed cell death in posterior wing disc cells. Thus loss of KDM5 led to increased apoptosis suggesting that KDM5 normally acts to repress IR-induced cell death. To understand how KDM5 controls apoptosis, we first asked whether the H3K4me3 demethylase activity of KDM5 was required for this function. To do that, we used flies specifically lacking KDM5-demthylase enzymatic activity. Dcp1 staining reveals no difference between wild type and demethylase inactive flies. Thus KDM5 acts as an anti-apoptotic factor independently of its demethylase activity.

We will continue to investigate the role of KDM5 in apoptosis and to define the precise mechanisms and target genes by which KDM5 controls the apoptosis. In particular, we are trying to identify KDM5-regulated genes involved in the IR-induced apoptotic.

690C DNA methylation is dynamic and undergoes changes during *Drosophila* development. *Saniya Deshmukh*, Varada Abhyankar, Chitra Panikar, Deepti Deobagkar. Department of Zoology, S. P. University of Pune, Maharashtra, India 411007.

A methylation is a conserved epigenetic modification which contributes to the plasticity in a genome; its role is commonly studied in mammals and eusocial insects. The genome of *Drosophila melanogaster* possesses very low levels of 5-methylcytosine. *Drosophila* undergoes a complete holometabolous development from the embryonic stage to the mature adult stage; making it an interesting system to explore the potential biological role of this low-level cytosine methylation in a stage- specific manner.

In our analysis, we have compared life cycle stages of *Drosophila melanogaster* using a wide range of high-throughput tools from methylation-sensitive restriction enzymes to antibodies in combination with microarray and immunoprecipitation-based analysis in order to identify this cytosine modification. We report that DNA methylation changes in a stage and gene-specific manner in *Drosophila melanogaster*. For instance, our analysis demonstrates that the zygotic genes and homeotic complex, implicated in embryonic patterning and development, are methylated in the adult and pupal stages, however, genes involved in crucial pathways like MAPK-signalling, are also methylated in the adult stage. This data emphasises on the possibly diverse role of non-CpG methylation in the coordination of chromatin dynamics, reprogramming and gene expression. Our work also suggests that despite preferential methylation of certain DNA motifs; there is an observed difference in their methylation status with respect to the stage of development under consideration.

Our study reflects upon the dynamic changes in DNA methylation in *Drosophila* with respect to the specificity of sequence, correlation with gene expression and its probable role in development.

691A The Tet-dependent epitranscriptome: the role of 5-hydroxymethyl Cytosine (5hmrC) in Development. *Joseph Kramer*[1], Fei Wang[2], Badri Singh[2], Tarun Uppalati[2], Andria Linfante[2], Steward Ruth[2]. 1) Department of Pathology and Laboratory Medicine, RBHS -- Rutgers University, Piscataway, NJ; 2) Waksman Institute, Molecular Biology and Biochemistry Rutgers University, Piscataway, NJ.

The *Drosophila* Tet protein (dTet) is the sole *Drosophila* homologue of the vertebrate methyldioxygenases TET1, TET2, and TET3. Vertebrate TET proteins are well established as demethylation activities for a common vertebrate

DNA base, 5-methylcytosine, and ultimately lead to the replacement of 5mC with unmodified cytosine. This process counteracts the silencing associated with methylated Cs. Recently, we described a novel activity for the TET gene family implicating members in the conversion of 5mC in RNA (5mrC) to 5hmrC. dTET expression and the 5hmrC mark peak at 6-12 hours in embryos and is most abundant in the peripheral and central nervous system and dorsal vessel. Our study focuses on the identification the specific mRNA targets of dTET-dependent hydroxymethylation during embryonic development and on the mechanism by which hydroxymethyl marks are placed. Further we are investigating the requirement of Tet in *Drosophila* development and physiology. Homozygous *Tet^{null}* animals die at late pupal stages without displaying a strong phenotype. Our results do not implicate Tet in the development of specific tissues or cells, but rather suggest a requirement in the function of nerve and possibly muscle cells.

692B *Drosophila mir-274* regulates invasive cell migration in wing epithelia *C. Chang*[1], F. Chang[1], J. Li[2], C. Chen[2], Y. Tsai[1]. 1) Department of Life Science, Tunghai University, Taichung, Taiwan; 2) Institute of Molecular and Genomic Medicine NHRI, Maoli, Taiwan.

MicroRNA is composed of 21 to 23 ribonucleatides and it regulates target genes expression at post-transcriptional level. MicroRNAs involve in developmental processes and cancer formation. *Drosophila* is a good genetic system and has powerful genetic tools. Previously, our laboratory found that expressing *mir-274* in *Drosophila* wing epithelial cells induced epithelial cell migration. In this study, we study the molecular mechanism of *mir-274* in epithelial cell migration *in vivo*. We found that expression of *mir-274* induced epithelial-mesenchymal transition like cell migration. Expression of *mir-274* induced Rab11 and its downstream gene, integrin, in wing discs. Reducing Rab11 dosage suppressed *mir-274*-induced cell migration. The result suggests that *mir-274* induced cell migration through Rab11. To understand endogenous roles of *mir-274*, we employed the Transcription activator-like effector nucleases (TALEN) technology to generate *mir-274* mutants. The *mir-274$^{6-3}$* mutant had low *mir-274* expression in adult fly. Recently, it is reported that *mir-274* mutant shows defect in fly blood-brain barrier (BBB). BBB is composed of glial cells around CNS in *Drosophila* brain. We found *mir-274$^{6-3}$* also showed severe BBB defect. In the future, we will further study the potential mechanism of *mir-274* in cell migration in *Drosophila.*

693C Understanding phased primary-piRNA biogenesis in *Drosophila* *Daniel Tianfang Ge*[1,5], Cindy Tipping[1,2,5], Wei Wang[3,5], Phillip D. Zamore[1,2,4,5]. 1) RNA Therapeutics Institute; 2) Howard Hughes Medical Institute; 3) Program in Bioinformatics and Integrative Biology; 4) Department of Biochemistry & Molecular Pharmacology; 5) University of Massachusetts Medical School, Worcester, MA.

In the *Drosophila* ovary, PIWI-interacting RNAs (piRNAs) suppress transposon expression, ensuring female fertility. The RNA helicase Armitage (Armi) and the endoribonuclease Zucchini (Zuc) are both required for phased primary-piRNA biogenesis, in which a long single-stranded piRNA precursor is processed into multiple consecutive piRNAs (phasing). The mechanism underlying phasing remains poorly understood. We find that the Armi ATPase activity is required to produce phased piRNAs. Armi defective in ATP hydrolysis fails to colocalize with Zuc on the outer surface of mitochondria, whereas catalytically inactive Zuc fails to release Armi from the mitochondrial surface. To characterize the protein-RNA complex responsible for phasing, we engineered the endogenous Zuc gene to incorporate an affinity tag, then isolated Zuc-containing ribonucleoprotein complex from ovaries by anti-tag immunoprecipitation. We identified Armi as a transient component of this complex. We are currently analyzing additional protein and RNA components of the Zuc complex to better understand the molecular basis for piRNA phasing.

694A The interplay of Piwi and heterochromatin proteins in transposable element silencing in the germline. Anastasia Stolyarenko, *Mikhail Klenov*. Dept Molecular Genetics of Cell, Institute of Molecular Genetics, Moscow, Russian Federation.

Transposable element (TE) repression is critical for germline genome stability and fertility of animals, including *Drosophila melanogaster*. The piRNA-binding protein Piwi was shown to induce transcriptional repression of TEs in *Drosophila* ovaries. However, the interplay of Piwi and the main heterochromatin protein HP1a in this process remains not fully understood. We took advantage of a genetic approach of combining in one genotype germline knockdowns (KDs) of Piwi and HP1a and comparing them with single Piwi and HP1a KDs in the same genetic background via RNA-seq. We divided TEs according to their response to Piwi and HP1a KDs into four groups. The first two groups consist of TEs that are controlled by only one of the two proteins – Piwi-dependent and HP1a-dependent groups. We found that silencing of HP1a-dependent TEs also require heterochromatic protein Su(Var)3-9. The third TE group requires both Piwi and HP1a for repression, which points to their participation in a single pathway. Finally, the fourth group of TEs is derepressed only upon double Piwi HP1a KDs, but not upon single Piwi or HP1a KDs, which implies double control by Piwi and HP1a independently. Interestingly, according to ChIP-seq data, these TEs are highly enriched in H3K9me3 mark, which is indicative of their localization in heterochromatin. Our results show that Piwi-induced silencing and Piwi-independent repression by heterochromatic proteins represent two distinct systems that silence transcription of TEs in the ovarian germline. These two systems can act autonomously or in

association with each other to repress an element. Supported by Russian Foundation for Basic Researches (16-04-01524).

695B Co-dependent assembly of piRNA cluster chromatin and piRNA precursor RNPs in *Drosophila* Ovaries *GEN ZHANG*[1], Shikui Tu[2], Zhiping Weng[2], William Theurkauf[1]. 1) Molecular Medcine, UMass Medical School, Worcester, MA; 2) Program in Bioinformatics and Integrative Biology, UMass Medical School, Worcester, MA.

The piRNA pathway has a conserved function in silencing transposons and maintaining genome integrity in the germline. In *Drosophila* ovaries, piRNAs are generated from source loci called piRNA clusters, which are distinguished from other genomic regions by binding of Rhino, an HP1a homologue, and its accessory proteins Deadlock and Cutoff. Expression of precursor RNAs from piRNA clusters also requires the TREX complex (THO complex and UAP56), and piRNA cluster transcripts associate with both the THO complex and UAP56. We show that *rhino* mutations, which lead to increased cluster transcript splicing, block UAP56 recruitment to cluster transcripts, and that a point mutation in UAP56 that disrupts piRNA production also significantly reduces binding to the THO complex. Mutation in *thoc7*, which encodes a THO complex subunit, disrupts piRNA production, transposon silencing, and lead to increased cluster transcript splicing. The Rhino loses localization at piRNA cluster chromatin in *thoc7* mutant. Rhino thus promotes assembly of piRNA precursor complexes containing UAP56 and THO, and these complexes suppress cluster transcript splicing and promote assembly of cluster chromatin.

696C Analyzing the expression and RNA Localization of long non-coding RNAs in *Drosophila* adult Gonads *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, ON, Canada.

The number of non-coding genes exceeds the number of coding genes in many species - including humans. We are just starting to understand the biological roles of a hand full of these genes in different species. In *Drosophila* we and others have shown that long-non coding RNAs -lncRNAs- show intricate tissue and sex-specific expression patterns. In a recent study we concluded that: 1) most mRNAs are subcellularly localized; 2) most of the lncRNAs that we analyzed are expressed during development and; 3) when expressed, almost all lncRNAs are sub-cellularly localized, the majority in cytoplasmic locations.

In this project we aim to analyze the expression and sub-cellular RNA localization of lncRNAs in adult testes and associated tissues (accessory glands, seminal vesicles and ejaculatory ducts). We found so far that more than 50% of lncRNAs analyzed are expressed in these tissues, with the exception of the ejaculatory duct. When expressed, 95% are subcellularly localized, with expression levels and sub-cellular distributions both changing dramatically in rich and depleted diets. In order to link expression and function of these genes, we are in the process of analyzing a sub-group of lncRNAs that have been shown to be essential for male fertility and with particularly interesting expression patterns.

697A Temporal regulation of the Smaug RNA-binding protein in the early embryo *Wen Xi Cao*[1], Alexander Marsolais[2], Matthew Cheng[2], Najeeb Siddiqui[1], Hua Luo[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 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 hundreds of maternal transcripts during the maternal-to-zygotic transition (MZT). Both *smg* mRNA and SMG protein are cleared from somatic cells towards the end of the MZT. We have shown that degradation of *smg* mRNA requires the RNA-binding proteins, Brain Tumor, Pumilio, Nanos and Argonaute 1. SMG protein instability is directed by a 233 amino-acid region C-terminal to SMG's RNA-binding domain. When this region is deleted, SMG protein over-accumulates and persists beyond blastoderm cellularization. Higher resolution mapping has revealed redundant instability elements within the C-terminus. Why is SMG cleared late in the MZT? A subset of SMG's maternal mRNA targets is re-expressed zygotically. We, therefore, hypothesized that clearance of SMG is necessary to permit these transcripts to accumulate. Indeed, embryos expressing persistent SMG protein show reduced levels of several of these transcripts. Current experiments are focused on detailed mapping of the instability elements in the SMG C-terminus; identifying the mechanisms and factors that confer instability; and global analysis of the effects of persistent SMG on zygotic gene expression during the MZT.

698B Role of *Dscam1* 3' UTR extension in axon guidance Ryan Peterson, Zhiping Zhang, Bong Min Bae, Henry Ng, Jung Hwan Kim, Tom Kidd, *Pedro Miura*. Dept of Biology, University of Nevada, Reno, Reno, NV.

More than half of all genes in species from *Drosophila* to human undergo Alternative PolyAdenylation (APA). Neuronal tissues are enriched for usage of transcripts with extended alternative 3´ untranslated regions (3´ UTRs),

which are products of APA. Specific functions for these longer 3´ UTR transcripts are largely unknown. The *Dscam1* gene plays a major neurodevelopmental role in *Drosophila*. It expresses alternative length 3´ UTR isoforms in addition to expressing many thousands of alternatively spliced isoforms. Dscam1 protein expression is mostly restricted to neurons and in particular axons of the CNS; surprisingly, fluorescence in situ hybridization revealed that short 3´ UTR *Dscam1* transcripts were most highly expressed in glial cells at the embryo midline. In contrast, transcripts harboring the extended 3´ UTR were exclusively localized to neurons, suggesting that these isoforms are required for effective translation in the CNS. We identified the RNA-binding protein Elav as the protein responsible for biogenesis of extended *Dscam1* transcripts in neurons. To investigate the functional relevance of *Dscam1* extended 3´ UTR isoforms, we employed CRISPR gene editing to eliminate their expression while leaving short 3´ UTR isoforms intact. These flies displayed severe impairments in longitudinal axon guidance, ellipsoid body formation, and locomotion. The extended 3´ UTR might impart regulatory elements that promote Dscam1 translation in neurons and/or might be selectively associated with particular upstream alternative protein-coding exons. We have identified multiple additional genes involved in axon guidance to also express Elav-regulated extended 3´ UTRs; thus, 3´ UTR extension might constitute a broad mechanism controlling this key neurodevelopmental event.

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699C Structure-Function Analysis of *gurken* IRES Activity *Joshua Blundon*[1], Brian Guy[1], Anthony Tardibuono[1], Ramses Rodriguez[1], Cory Emborski[2], Matthew Fountain[2], Scott Ferguson[1]. 1) Biology, The State University of New York at Fredonia, Fredonia, NY; 2) Biochemistry & Chemistry, The State University of New York at Fredonia, Fredonia, NY.

Gurken (*grk*) is an EGFR ligand that establishes dorsal-ventral and anterior-posterior patterning in oocyte development in *Drosophila melanogaster*. Spindle-B is required for for double stranded DNA break repair during homologous recombination in meiosis. Females that are mutant for *spn-BBU*, have persistent DNA double stranded breaks during early oogenesis and this causes a ventralization of the eggshells due to a lack of Grk at later stages. This accumulation of DNA double stranded breaks cause the activation of the ATR/Chk2 meiotic checkpoint and subsequent inactivation of Vasa by phosphorylation. Vasa is an RNA helicase that is required for *grk* translation. We have found that *spn-BBU* mutants that are subject to dietary restriction lay more wild type eggs reflecting the return of Grk expression. We hypothesize that the *grk* mRNA has an Internal Ribosomal Entry Site (IRES) that initiates translation via recruitment of the ribosome independently of the 5' cap. Using an *in vitro* translation dual luciferase assay, our current data suggests the IRES is located in the *grk* 5'UTR. This assay allows us to control the cap-binding status by adding free competitor cap. In this assay, we have shown that *grk* translation is resistant to repression by excess free cap. Using a series of deletion mutants, we have identified regions required for IRES activity *in vitro*. We have also performed a CRISPR mutagenesis screen on the endogenous *grk* 5' UTR and found some InDel alleles that disrupt IRES activity. To determine the structures that are required for IRES translation, we performed a SHAPE experiment on the *grk* 5' UTR. In combination with the mutagenesis study, we have begin to identify the structures that facilitate IRES function.

700A Cytoplasmic polyadenylation of *grk* mRNA regulates the spatial distribution of Grk protein during oogenesis *Amanda Norvell*, Teddy Kozlowski, Krista Budinich. Dept Biol, Col of New Jersey, Ewing, NJ.

Precise spatial and temporal protein distribution is crucial for cellular function and inappropriate protein localization can contribute to disease states. In *Drosophila melanogaster*, the coupled cytoplasmic localization of mRNA transcripts with translational repression of unlocalized mRNAs during oogenesis is an efficient mechanism to fine-tune protein distribution and prevent subsequent embryonic patterning defects. During oogenesis, the TGF-alpha like protein Gurken (Grk) is localized to the dorsal-anterior corner of the oocyte, and this distribution is required for proper patterning of the dorsal-ventral (D-V) axis of the egg and future embryo. *grk* transcripts are actively transported to the dorsal-anterior and those that are not yet localized are subjected to translational repression, mediated by the RNA-binding protein Squid (Sqd). During the later stages of oogenesis, *grk* mRNA is subjected to cytoplasmic polyadenylation by the polyA polymerase Wispy and the CPEB protein Orb. We have proposed a model in which the bulk of *grk* mRNA in the ovary is modestly adenylated, but during late oogenesis transcripts that are localized to the dorsal-anterior of the oocyte are hyperadenylated. We suggest that the hyperadenylation of localized *grk* mRNA is important in its translation at this stage of oogenesis, either because underadenylated *grk* mRNA cannot be translated in this location or because translational repression of unlocalized *grk t*ranscripts is mediated through alteration of the poly(A) tail length. In order to distinguish these models and to understand what conditions are required for *grk* mRNA hyperadenylation, we are measuring *grk* mRNA poly(A) tail profiles in late stage oocytes from females carrying mutations that disrupt D-V patterning during oogenesis, including *sqd*, *fs(1)K10*, *enc* and *vasa*.

701B Ras/Erk-signaling regulates tRNA synthesis and cell proliferation through RNA polymerase III repressor Maf1 in *Drosophila* *Shrivani Pirahas*, Byoungchun Lee, Savraj Grewal. Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada.

Ras signaling promotes growth and proliferation in many tissues throughout animal development. An important challenge is to identify how the Ras-Erk pathway alters cellular metabolism to drive growth. Here we report on the control of tRNA synthesis as growth effector of EGF/Ras/Erk signalling in *Drosophila.* I find that overexpression of oncogenic Ras (Ras^{V12}) leads to increased mRNA translation and protein content in *Drosophila* S2 cells, suggesting that Ras may promote growth through enhanced protein synthesis. The conventional view is that the Ras pathway functions by controlling translation initiation factor activity. However I have identified an alternate mechanism involving control of tRNA synthesis. My data suggest that overexpression of Ras^{V12} or the activated versions of EGFR and the Raf1 in wing imaginal discs increases tRNA synthesis. Similarly, expression of Ras^{V12} in S2 cells increases tRNA levels, while blocking Ras/Erk signaling using the MEK inhibitor, U0126 or RNAi reduces tRNA synthesis. We previously identified the RNA polymerase III (Pol III) factor, Brf, as regulator of cell and tissue growth in *Drosophila*. Here we show that knockdown of either Brf blocks the effects of Ras signaling on growth and proliferation in larval wing imaginal discs, adult midgut progenitor cells and adult intestinal stem cells. Several transcription factors have been shown to link Ras signaling to changes in mRNA expression and growth. I have identified Myc is required but not sufficient for Ras-induced cell proliferation and growth through tRNA synthesis. Previously we have shown that TOR signaling regulates protein synthesis through RNA Pol III repressor, Maf1. I found that Maf1 RNAi increases tRNA synthesis in S2 cells. In addition, the decrease in tRNA synthesis induced by the MEK inhibitor is blocked in the presence of Maf1 RNAi suggesting Maf1 is downstream of Ras signaling pathway. My data point to control of tRNA synthesis possibly through Maf1 as a new mechanisms by which Ras signaling enhances protein synthesis to promote cell and tissue growth.

702C Long term *ex vivo* culture and live imaging of *Drosophila* larval imaginal discs *CHIA-KANG TSAO*[1,2], Hui-Yu Ku[1,2], Yuan-Ming Lee[1,2], Yu-Fen Huang[1,2], Y. Henry Sun[1,2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) 2.Institute of Genomic Sciences, National Yang-Ming University, Taipei, Taiwan.

Continuous imaging of live tissues provides clear temporal sequence of biological events. The *Drosophila* imaginal discs have been popular experimental subjects for the study of a wide variety of biological phenomena, but long term culture that allows normal development has not been satisfactory. Here we report a culture method that can sustain normal development for 18 hours and allows live imaging. The method is validated in multiple discs and for cell proliferation, differentiation and migration. However, it does not support disc growth and cannot support cell proliferation for more than 7 to 12 hr. We monitored the cellular behavior of retinal basal glia in the developing eye disc and found that distinct glia type has distinct properties of proliferation and migration. The live imaging provided direct proof that wrapping glia differentiated from existing glia after migrating to the anterior front, and unexpectedly found that they undergo endoreplication before wrapping axons, and their nuclei migrate up and down along the axons. UV-induced specific labeling of a single carpet glia also showed that the two carpet glia membrane do not overlap and suggests a tiling or repulsion mechanism between the two cells. These findings demonstrated the usefulness of an *ex vivo* culture method and live imaging.

703A Recent efforts to map mutations in Bloomington stocks *Lily Kahsai Golden*, Annette Parks, Ellen Popodi, Kathy Matthews, Thomas Kaufman, Kevin Cook. Department of Biology, Indiana University, Bloomington, IN.

The Bloomington Stock Center collection contains many stocks with mutations that have not been associated with annotated genes. We have been mapping these mutations in an effort to make them more useful to the Drosophila research community. We will present data from three efforts. First, we have examined recessive lethal and sterile mutations on P element insertion chromosomes to determine if they are associated with the sites of P insertions or if they map elsewhere. Second, we have used molecularly defined chromosomal deletions and previously characterized single-gene mutations to map mutations with lethal, sterile and visible phenotypes to minimal chromosomal intervals or, where possible, to single genes. Third, we have used whole-genome sequencing to identify candidate genomic lesions and have verified that they are associated with lethal and visible mutations with follow-up complementation tests. By characterizing mutations in existing stocks, we hope to provide novel alleles of genes and to eliminate redundant stocks.

704B CRISPR/Cas9 survey of Drosophila modENCODE cell lines *Andrew Zelhof*, Lei Gong, Vanessa Worthy, Johhny Roberts. Drosophila Genomics Resource Center, Indiana University, Bloomington, IN.

The Drosophila Genomics Resource Center maintains over 100 stable cell lines and these cell lines have become an integral part of the toolkit for Drosophila research. In particular, there are 25 Drosophila melanogaster cell lines (modENCODE cell lines) reported in Cherbas et al., 2011 that have been characterized by whole-genome tiling microarray analysis of total RNA, permitting researchers to choose the most appropriate cell line for investigations into gene function and cellular biology. Furthermore, with the advent of CRISPR/Cas9 the ability to manipulate the genome of these cells has decreased the reliance on transient transfections and increased the utility of stable cell lines. Nonetheless, CRISPR/Cas9 manipulations have been limited to only a few cell types and thus there is a need

to provide a systematic survey of the ability to manipulate as many different lines as possible. Therefore, here we will describe our efforts to establish a baseline/minimum set of conditions applicable to all modENCODE cell lines for CRISPR-Cas9 manipulations and reveal and note potential differences between cell lines to consider in planning experiments.

705C A Genetic Toolkit for Dissecting Dopamine Circuit Function *T. Xie*[1], M. Ho[1], Q. Liu[1], C Lin[2], S. Chin[2], W. Horiuchi[1], L. Jiang[3], B. White[3], C. Potter[2], M. Wu[1,2]. 1) Neurology, Johns Hopkins University, Baltimore, MD; 2) Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Molecular Biology,National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

Dopamine (DA) plays a key role in regulating animal behaviors, including feeding, learning/memory, courtship behavior, reward processing, and sleep. Fruit flies exhibit a number of these DA-dependent behaviors, but a comprehensive toolkit for dissecting the specific DA circuits underlying these behaviors is not currently available. To address this gap, we have created a series of transgenic *Drosophila* lines to facilitate the targeting and manipulation of small subsets of DA neurons. There are ~125 DA neurons in adult fly brains, and we chose 10 different promoter sequences that target distinct subsets of these cells. Using these promoter sequences, we generated and characterized Gal80, Gal4-AD, and Gal4-DBD transgenic lines. To annotate the precise DA cells labeled by all 90 Gal4-AD/Gal4-DBD combinations, we are performing brain registration to a standard brain and then aligning these images to individual DA neurons identified from the FlyCircuit database (Chiang et al., 2010). In order to further refine the intersectional patterns of these Gal4-AD/Gal4-DBD combinations, we also used these 10 promoter sequences to create “Killer zipper” (KZ) lines. KZ binds to and inhibits the split-Gal4 transcription factor, analogous to Gal80 inhibition of Gal4 (Luan et al., unpublished data). In addition, to aid the functional manipulation of DA signaling in flies, we have generated a novel *UAS-tyrosine hydroxylase-miRNA* line that specifically targets neuronal TH, as well as new miR lines targeting all 4 DA receptors in flies. We will present the current state of our characterization, validation, and annotation of these lines.

706A Characterizing the genetic basis of behavioural isolation between *Drosophila melanogaster* and *Drosophila simulans* using CRISPR/Cas9. *J. A. Bielaska Da Silva*, A. J. Moehring. Biology Department, University of Western Ontario, London, Ontario, Canada.

Behavioral isolation is a prezygotic mechanism that keeps two different species reproductively isolated from each other due to differences in courting and mating behaviour. This form of reproductive isolation is usually determined by female preference, such as seen with the rejection behavior exhibited by *Drosophila simulans* females towards *D. melanogaster* males. Though female rejection behavior has been behaviorally characterized, the genes that contribute to this behavior are still largely unknown. Previous research has identified a candidate gene for female rejection behavior: *not my type* (*nmt*). Females with the *D. melanogaster* allele are receptive towards *D. melanogaster* males, while females that have the *D. simulans* allele reject *D. melanogaster* males. Here, I used CRISPR/Cas9 to disrupt expression of *nmt* in either *D. melanogaster* or *D. simulans* to allow for the generation of interspecies female hybrids expressing only a single species-specific allele of *nmt* in order to confirm this gene’s role in behavioral isolation. A reciprocal hemizygosity test will then be used to compare the behavioral phenotype of the hybrid females: these females will be genetically identical except at the *nmt* locus, where they will only express the *D. simulans* or *D. melanogaster* allele. This experimental approach will help to better understand the genetic basis of speciation.

707B Quantification of Drive Efficiency and Resistance Allele Creation in CRISPR Gene Drives *Jackson Champer*[1,2], Riona Reeves[1,2], Suh Yeon Oh[1,2], Chen Liu[1,2], Jingxian Liu[1,2], Andrew G. Clark[1,2], Phillip W. Messer[1]. 1) Department of Biological Statistics & Computational Biology, Cornell University, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

CRISPR-based gene drive constructs direct the insertion of themselves into the homologous site of wild-type alleles so that heterozygotes transmit them at much higher than Mendelian ratios. Gene drives promise a diverse array of applications, most notably suppression of vector-borne human diseases. Initial studies with Cas9 driven by the *Vasa* promoter have shown promise in *Drosophila* and mosquitoes. One drawback of current gene drive systems is their propensity to generate resistance alleles by non-homologous end joining. These alleles have an altered target sequence, preventing their cleavage by Cas9 and conversion to the gene drive allele. We sought to design a system with reduced rate of formation of resistance alleles by using Cas9 driven by the *Nanos* promoter, which have more germline-specific expression than *Vasa*. Thus, we designed two variants of CRISPR gene drives and incorporated these into *Drosophila melanogaster* lines. One of these targets the X-linked *yellow* gene and contains Cas9 driven by the *Nanos* promoter. Our other drive system targets the *yellow* gene promoter and contains Cas9 driven by the *Vasa* promoter. The *Nanos*-based gene drive resulted in 83% transmission from heterozygote mothers, while the *Vasa* drive reached 74%. While lower than the *Vasa* Drive, resistance allele creation nevertheless reached 11%

in the *Nanos* drive for maternal alleles and 18% post fertilization in paternal alleles. An additional 22% of flies experienced post-fertilization mosaic conversion of paternal alleles to resistance alleles. Sequencing of resistance alleles revealed mostly small deletions at the CRISPR target site. In contrast to a previous study, we found in our system no post-fertilization events in which a wild-type allele was converted to a gene drive allele. Overall, the relatively rapid formation of resistance alleles continues to impose a severe limitation on the effectiveness of gene drives applied to highly diverse natural populations. Future studies will need to reduce the level of resistance allele creation for gene drives to be viable alternatives for vector control.

708C Investigating parameters affecting efficiency of CRISPR guide RNAs *James Kwon*, Greg Beitel. Molecular Biosciences; Weinberg College of Arts and Sciences, NORTHWESTERN UNIVERSITY, EVANSTON, IL.

The recent development of the CRISPR-Cas9 system for genome editing has revolutionized our ability to modify the endogenous DNA sequences. However, many practical details of gene editing remain unclear, including the number and design of guide RNAs. Preliminary tests of inserting a dsRed marker into a variety of loci using either one or two guide RNA plasmids and a repair template yielded repair efficiencies ranging from 5% to 20%, but did not reveal an obvious correlation between efficiency and the use of one versus two guide RNAs. However, there may be differences in the frequency of insertion versus repair events when using one versus two guide RNAs. In *C. elegans*, it has been reported that guide RNAs containing 3'GG PAM sequences are much more efficient at targeting than non-3'GG-containing guide RNAs (Farboud and Meyer, Genetics 2015). However, our preliminary results using the *Drosophila white* locus suggest that, 3'GG-containing guide RNAs do not consistently yield higher targeting frequencies than non-3'GG-containing guide RNAs. Updated data will be presented at the meeting.

709A New from the TRiP: transgenic *Drosophila* sgRNA libraries for gene overexpression and knockout by CRISPR-Cas9 *Jonathan Zirin*[1], Ben Ewen-Campen[1], Yanhui Hu[1], Aram Comjean[1], Luping Liu[1], Rong Tao[1], Christians Villalta[1], Donghui Yang-Zhou[1], Ryan Colbeth[1], Michael Pyle[1], Stephanie Mohr[1], Lizabeth Perkins[1], Verena Chung[2], Shu Kondo[3], Jianquan Ni[4], Norbert Perrimon[1,5]. 1) Dept of Genetics, Harvard Medical School, Boston, MA; 2) College of Science, Northeastern University, Boston, MA; 3) Invertebrate Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; 4) Tsinghua Fly Center, Tsinghua University, Beijing, China; 5) Howard Hughes Medical Institute, Boston, MA.

The Transgenic RNAi Project (TRiP) is an in vivo functional genetics platform that has generated over 12,000 *Drosophila* RNAi stocks for the research community (Perkins et al., 2015 Genetics. 201:843-52). Recently the TRiP has transitioned from predominantly RNAi fly stock production to development of two new large scale in vivo resources based on CRISPR technology. Each stock in the TRiP CRISPR Overexpression collection (TRiP-OE) expresses two sgRNAs that target upstream of a gene transcription start site. Crossing TRiP-OE stocks to a Gal4 line expressing catalytically dead Cas9 fused to an activator domain (dCas9-VPR, Chavez et al., 2016 Nature Methods. 13:563-7) induces activation of the target gene. In the TRiP CRISPR Knockout collection (TRiP-KO), each stock expresses one or two sgRNAs targeting the coding sequence of a gene or genes. Crossing TRiP-KO stocks to a Gal4 line expressing Cas9 induces cleavage of the target site, non-homologous end joining (NHEJ) repair and insertion/deletion mutations (indels) in both germline and somatic tissue. To facilitate studies with these collections, we also produced a toolbox of Gal4/Gal80ts/UAS stocks that allow spatial and temporal expression of Cas9 or dCas9-VPR. Initial characterization of TRiP-OE and -KO lines indicate a large proportion are functional, providing powerful new tools for gene activation and genome engineering. Stock production thus far has focused on mini-libraries of gene categories (eg. orthologs of human disease associated genes and rate-limiting enzymes), but we also accept nominations from the community. Through our sgRNA Stock Tracking System (http://www.flyrnai.org/tools/grna_tracker/), researchers can search existing stocks by gene identifier or by stock number, as well as nominate genes for TRiP-OE or TRiP-KO production. As with our earlier RNAi libraries, the new transgenic CRISPR stocks are sent to the Bloomington Drosophila Stock Center for distribution to the fly community.

710B EC-Tagging Allows Cell Type-Specific RNA Analysis *Mohamed Aboukilila*[1], Naoki Hida[1], Rakesh Paul[2], Marc Greenberg[2], Mike Cleary[1]. 1) Quantitative and Systems Biology Graduate Group, University of California, Merced, Merced, CA; 2) Department of Chemistry, Johns Hopkins University, Baltimore, MD.

Purification of cell type-specific RNAs from tissues or whole organisms remains a significant technical challenge. One solution involves metabolic tagging of RNAs via incorporation of a modified nucleotide and subsequent purification of tagged RNAs. RNA tagging via incorporation of 4-thiouracil in cells expressing transgenic uracil phosphoribosyltransferase (UPRT), a method known as TU-tagging, has been applied in *Drosophila* and mice. However, TU-tagging can have limited specificity due to endogenous UPRT activity. Here we describe an alternative method of RNA tagging that requires the activity of two enzymes: cytosine deaminase (CD) and UPRT. We found that the sequential activity of these enzymes converts a modified cytosine, 5-ethynyl-cytosine (5EC), to 5-ethynyl-uridine monophosphate (5EU) that is subsequently incorporated into nascent RNAs. The ethynyl

group allows "click chemistry"-based detection and purification of tagged RNAs. We show that "EC-tagging" of RNA only occurs in cell lines and *Drosophila* engineered to express CD and UPRT (or a CD:UPRT fusion), with no detectable RNA tagging in control cells or control animals. We demonstrate the sensitivity and specificity of EC-tagging by obtaining cell type-specific mRNA expression data from *Drosophila* larvae. EC-tagging combined with body wall dissection allowed purification of RNA from a very small population of PNS multidendritic neurons (using *ppk-gal4* to drive *UAS-CD:UPRT* expression). These experiments revealed enrichment of neural transcripts and depletion of muscle transcripts by RT-qPCR. EC-tagging also allowed purification of neural-specific RNA from whole larvae, without the need for any physical dissection. Microarray analysis of purified neural RNA further demonstrated the sensitivity and specificity of EC-tagging and revealed novel neural gene expression properties. EC-tagging provides several advantages over existing techniques for cell type-specific RNA analysis and should prove useful for various studies in *Drosophila.*

711C   Ilastik- and Matlab-based computational tools for analyzing biological tubes in 3-D   *R. Yang*[1], E. Li[1], M. Mani[1,2], G.J. Beitel[1]. 1) Dept of Molecular Biosciences, Northwestern University, Evanston, IL; 2) Dept of Engineering Sciences and Applied Mathematics, Northwestern University, Evanston, IL.

The architecture of biological tubes must be tightly regulated for an organism's survival. Understanding the mechanisms of tube size control requires the ability to quantify many aspects of tube structure such as tube length and diameter, and cell shape and orientation. Our objective was to develop computational tools that could robustly measure differences in morphology of the Drosophila trachea. Although programs to analyze planar epithelia exist, they are unable to process the more complex problem of 3-dimensional tubes or to measure cells on highly curved surfaces. We therefore developed a workflow using Matlab and an open source segmentation program called Ilastik to analyze tubular epithelia. In this workflow, the tracheal dorsal trunk and the apical surface of the tracheal cells were segmented from confocal images using Ilastik. Segmented data were imported to Matlab, and a marching cubes algorithm was used to define the centerline down the middle of the tube and detect branches. Apical cell outlines were then mapped onto the tube surface. Tubes were analyzed for a variety of parameters including branch length, lumenal volume, cross-sectional regularity, cell size, and cell orientation relative to the local tube centerline. Analysis of *src42A*$^{26-1}$ mutants using this workflow identified tube length defects and cell orientation defects comparable to those reported by Nelson et al. (2012) and Förster and Luschnig (2012). To better visualize patterns of cell and subcellular organization, we are extending the code to "unroll" the tracheal tubes onto 2-D surfaces. Preliminary analysis of maps of the orientation vectors of tracheal cells suggests that there are anterior-posterior differences in cell orientations along the length of the dorsal trunk.

712A   Large-scale functional genomics in *Drosophila*: Reagents and tools for assays, analysis, and integration.   *Stephanie Mohr*[1], Yanhui Hu[1], Aram Comjean[1], Benjamin Housden[1], Raghuvir Viswanatha[1], Katarzyna Sierzputowska[1], Gabriel Amador[1], Charles Roesel[1,2], Verena Chung[1], Lizabeth Perkins[1], Jonathan Zirin[1], Norbert Perrimon[1,3]. 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) Marine Science Center, Northeastern Univ, Nahant, MA; 3) Howard Hughes Medical Institute, Boston, MA.

The *Drosophila* RNAi Screening Center (DRSC) provides access to reagents, assays, and other support for large-scale functional genomics screens in *Drosophila* cultured cells. The DRSC makes regular updates to offer the state-of-the-art in functional genomics screening with RNAi and CRISPR technologies; bioinformatics analysis; and data management, analysis, and integration. We have recently expanded support in three areas: reagent libraries, cell screen technologies, and bioinformatics tools. ***New cell RNAi library.*** New reagent resources include CRISPR modified cell lines and a new RNAi library for cell-based screens that targets fly orthologs of human proteins for which there exist FDA-approved drugs or compounds. ***New screen technologies.*** Three new screen technologies were added or are under development. First, we replaced an outdated imaging system with a new automated confocal fluorescence imaging system, available to all visiting researchers at the DRSC in Boston, MA. Second, we now offer access to the variable dose analysis (VDA) approach, which combines RNAi with FACS to deconvolve a dose-curve effect of RNAi reagents on cell number. VDA provides a sensitive assay for synthetic lethal screening. We are collaborating with labs to apply the approach in the context of gene discovery relevant to cancer cell biology. Third, we are exploring the use of large-scale CRISPR single guide RNA (sgRNA) libraries for pooled format cell-based screens, including to identify essential genes and in selection assays. Initial results suggest this offers a powerful new platform amenable to a wide range of assays that complement what is available using arrayed RNAi screen approaches. ***New and updated online tools.*** We recently expanded support for our popular DRSC Integrated Ortholog Prediction Tool (DIOPT) by supporting multi-species searches and providing multi-protein alignments. We also added the *Drosophila* Gene Expression Tool (DGET), which facilitates mining of modENCODE and other RNAseq data at a convenient, batch search-compatible web interface, and made updates to the Find CRISPRs sgRNA genome browser-based search tool. As presented at our newly updated DRSC/TRiP Functional Genomics Resources website (http://fgr.hms.harvard.edu/), our resources provide the community with a wide array of reagents,

assays, cells, and online software tools for small and large-scale projects that can be performed either on-site at our facility or off-site in your own lab.

713B flyDIVaS v2.0: An updated comparative genomics resource for lineage-specific divergence and selection in Drosophila *Craig Stanley*, Steven Weaver, Sergei Pond, Rob Kulathinal. Biology, Temple University, Philadelphia, PA.

flyDIVaS is a comparative genomics database of Drosophila divergence and selection, based on the latest genomic assemblies, curated FlyBase annotations, and OrthoDB orthology calls from 12 species of Drosophila. Here we describe the next iteration, *flyDIVaS v2.0*, which incorporates significant advances to the original database. We update genome-wide inferences of selection using methodologies from HyPhy, a robust and alternative package of phylogenetic methods to detect adaptive change, and incorporate three different methodologies aimed at identifying gene-wide selection, episodic-selection, and lineage-specific selection. We further add new tools that allows the user to choose a lineage of interest from interactive phylogenies and alignments. These new functionalities enhance *flyDIVaS v2.0*'s role as an important portal to study evolutionary divergence and adaptation on a genome-wide scale.

714C Using UniProtKB to aid Drosophila research *Kate A Warner*[1], UniProt Consortium[1,2,3,4]. 1) European Bioinformatics Institute, Cambridge, United Kingdom; 2) Swiss Institute of Bioinformatics, Centre Medicale Universitaire, 1 rue Michel Servet, CH-1211 Geneva 4, Switzerland; 3) Protein Information Resource, Georgetown University Medical Center, 3300 Whitehaven St. NW, Suite 1200, Washington, DC 20007, USA; 4) Protein Information Resource, University of Delaware, 15 Innovation Way, Suite 205, Newark, DE 19711, USA.

The UniProt Knowledgebase (UniProtKB) provides the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional information. It facilitates scientific discovery by organising biological knowledge and enabling researchers to rapidly comprehend complex areas of biology. The Drosophila protein annotation program focuses on the expert annotation of characterised D. melanogaster proteins. Expert curation of a UniProtKB entry involves using a range of sources to collect scientific information on a protein and then manually assessing the quality of that data before integration into the entry. All relevant scientific publications are read in detail to extract information such as function, expression, protein-protein interactions, subcellular location and effects of gene disruption. This is combined with additional data from protein sequence analysis tools, other databases and automatic annotation systems. The quality of predicted and experimental data is critically assessed and all information in an entry is attributed to its original source, so that each entry provides a comprehensive review of the most current biological information for a protein. In addition, UniProtKB provides links to more than 150 other resources including FlyBase, enabling researchers to easily access complementary information in other databases. The current UniProtKB annotation process will be presented with emphasis on how to utilise UniProtKB to aid research. UniProt is updated every four weeks and can be freely accessed or downloaded from http://www.uniprot.org.

715A Developing M-TRAIL: A tool to track cell migration *in vivo* *Dong-Yuan Chen*, David Bilder. Dept. of Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA.

Cell migration is a key process for tissue morphogenesis and homeostasis across animal species. Live imaging *in vivo* or *ex vivo* allows mechanistic insights, but long-term observation can alter normal biology, and tools to track cell movements *in vivo* without perturbation have been lacking. We have taken advantage of the relationship between migratory cells and the extracellular matrix (ECM) to develop a tool called 'Matrix-labelling Technique for Real-time And Inferred Location (M-TRAIL)', which reveals cell migration histories in fixed tissues. By making single cell clones that overexpress GFP-tagged ECM proteins, past trajectories of motile cells can be mapped based on GFP trails deposited onto the basement membrane (BM). We applied M-TRAIL to track cell migration during *Drosophila* ovarian follicle rotation and subsequent cell movements, in both WT and mutant conditions, comparing *in vivo* migratory dynamics with those previously obtained using *ex vivo* culture. Interestingly, M-TRAIL demonstrates that follicles lacking the intracellular domain of the atypical cadherin Fat2, which were previously reported to fail in rotation, in fact rotate *in vivo* albeit at a reduced speed. As this genotype was proposed to uncouple follicle rotation from tissue elongation, the data show that a sole reliance on *ex vivo* live imaging can be insufficient. Together, our results demonstrate the utility of M-TRAIL in tracking and quantitating cell motility *in vivo* at cellular resolution; the principle may be applied to other tissues and organisms.

716B Method for Measuring Metabolism of Whole Larval Brains in *Drosophila* and Beyond *Mia Klekos*, Timothy Bosse, Marla Tipping. Providence College, Providence , RI.

It is important to study how metabolism is affected in disease in order to isolate potential therapeutic targets. One way to measure metabolism is by utilizing the Seahorse Biosciences XFe96 Flux Analyzer. Previously, the XFe96 analyzer has been used to measure the metabolism of plated cells and tissue punches. However, the most useful

analysis comes from measuring the metabolism of whole tissues/organs *in situ*. We have developed a method for measuring the metabolism of whole *Drosophila* larval brains using the XFe96. The main issue in measuring the metabolism of whole tissues is the tissue's ability to securely attach to the base of the well while being analyzed. With the assistance of collaborators, we have designed a capture screen to stabilize the tissue for metabolic measurement. We have observed predicted incremental increases in metabolic readouts when obtaining baseline measurements from one, two, or three larval brains per well. These results confirm that the capture screens are working effectively. With this confidence, we then obtained baseline measurements of larval brains of different wild type strains, which demonstrated similar metabolic rates. To further the investigation of metabolism *in situ*, we administered drugs to larva of varying genotypes, and observed differences in metabolic vulnerability in the brains of these animals. We believe that our developed method to study metabolic changes can be utilized to study the affects of metabolism in disease using whole tissues, by not only the fly community, but also in other model systems.

717C New stocks and resources at the Bloomington Drosophila Stock Center *Cale D. Whitworth*, Kathy A. Matthews, Kevin R. Cook, Annette L. Parks, Sam Zheng, Thom C. Kaufman. Bloomington Drosophila Stock Center, Department of Biology, Indiana University, Bloomington, IN.

The Bloomington Stock Center currently carries over 59,000 stocks, and in 2016 we shipped over 220,000 stocks to ~2,000 groups in 54 countries. In 2016 we added >800 TRiP stocks bringing the total number of UAS-RNAi stocks to over 12,000. For studying human health and disease, we more than doubled the number of UAS-human-cDNA lines bringing our total to ~700. We continued acquisition of both the Mi{MIC}-based Recombinase-Mediated Cassette Exchange lines and modERN lines, which both express tagged proteins under the control of native regulatory sequences. These ~1,000 lines allow for localization of tagged proteins as well as protein knockdown in a reversible, spatial, and temporal manner. We expanded our collection of binary overexpression system lines—such as GAL4/UAS, lexA/lexAop, and QF/QUAS—for use independently or in combination. These new lines include the 23 most popular GAL4 lines "HACKed" to express QF2 in the original GAL4 pattern. We acquired a variety of exciting new stocks including calcium and voltage sensors, organelle markers, stocks carrying defined strains of Wolbachia, and new CRISPR/Cas9 reagents. We will provide an overview of these and other new acquisitions and highlight some important sets of pre-existing and useful stocks. As always, we hope we will give you ideas for experiments or new ways to explore biological processes. We welcome all suggestions and questions, so come by and see us!

718A Induced Cessation of Feeding in *Drosophila Melanogaster* *Anthony T Salvato*, Chris Tat, Nazzy Pakpour. California State East Bay , Hayward , CA.

Drosophila suzukii is a crop pest in California that causes an estimated annual $500 million in crop damage. Traditional pesticides are not effective against *D. suzukii* because of their unique life cycle. While most fruit flies occupy rotting fruit during their larval and pupal stages, *D. suzukii* larval and pupal stages are found in ripening fruits. The females are equipped with a serrated ovipositor that cuts into the flesh of ripe fruit. This both injures the fruit and protects the offspring from pesticides during their development. Thus, there is a need for novel methods of control that can be used to manage crop destruction by *D. suzukii*.

One such method would be to disrupt the feeding of adult *D. suzukii* so that they are unable to produced eggs. Studies have shown that serotonin signaling is linked to feeding behavior. Metitepine is a drug that has been determined to be a potent anorectic drug that acts as a serotonin receptor antagonist (5-HT). Therefore, metitepine may be able to act as appetite suppressant of *D. suzukii,* subsequently reducing lifespan and egg laying production of adults. Our preliminary studies were conducted with the model organism, *D. melanogaster,* and showed that ingestion of metitepine reduced both egg deposition and lifespan. Subsequent studies will focus on repeating these results in *D. suzukii*.

719B Characterization of the Putative Sugar Transporter Encoding Genes, *SUT3* and *SUT4*, from *Drosophila melanogaster* *Stephanie Hrabar*, Mark Hiller. Goucher College 1021 Dulaney Valley Rd. Baltimore, MD 21204.

Sequencing of the *Drosophila melanogaster* genome revealed a group of genes termed *sut* genes that were annotated as sugar transporters, although the specific sugar transported by the gene products is not known. Genome wide expression studies curated by flybase (Gbrowse2) showed that two of the four genes, *sut3* and *sut4*, are only expressed in the late larva and pupa development stages and adult male. *sut1* and *sut2* are more widely expressed. The expression pattern suggests that *sut3* and *sut4* are testis-specific transporters. Using RT-PCR, we will confirm that both genes exhibit male-specific expression. We will also determine if *sut3* and *sut4* are expressed in the germline or depend on the germline for expression. Putative insertion mutations in each have been isolated by the Drosophila Gene Disruption Project, but do not result in any observed phenotype. We will determine if a putative insertion mutation interrupts *sut3* and *sut4* gene expression. To test if *sut3* and *sut4* are genetically redundant, we are creating a double mutant. Answering the basic biology questions of location of expression and mutant phenotypes will lay the groundwork for future studies, such as protein function

including the type of sugar transported. The initial characterization of the *sut1*, *sut3*, and *sut4* genes were performed as part of a Course Based Research Laboratory in Genetics. The benefits to undergraduate science education of Research Based Courses is documented and becoming more widespread. Here we discuss how these courses can lead to student-faculty collaborative research projects.

720C Inquiry-Based Laboratory Education Using *Drosophila* Molecular Methods *Jay Lusk*[1], Jan Gruber[1,3], Abil Saj[4], Nicholas Tolwinski[1,2]. 1) Division of Science, Yale-NUS College, Singapore, Singapore; 2) National University of Singapore Department of Biological Science, Singapore, Singapore; 3) National University of Singapore, Department of Biochemistry, Singapore, Singapore; 4) Genome Institute of Singapore, A-Star, Singapore, Singapore.

Given the need for science education to prepare students to ask key questions in science, while simultaneously developing key skills that are fundamental to biological research, we studied the implementation of an inquiry-based, practical approach to laboratory education using *Drosophila* molecular methods. This course was structured to encourage students to develop thoughtful and appropriate questions and think deeply about biology on the molecular level. The course was designed to prepare students with fundamental skills needed to conduct meaningful, original research in biology. Here we present the curriculum, which was structured to allow students to develop a deep understanding of *Drosophila* molecular methods by having them clone, recombine, and analyze genes of interest before evaluating them in *Drosophila* cell culture. We then evaluated the success of the course objectives through semi-structured interviews of students. We concluded that this course is an effective model to use *Drosophila* methods to develop deep skills in scientific inquiry.

721A The Genomics Education Partnership: A Bioinformatics Course-based Undergraduate Research Experience *J Sanford*[1], M Burg[2], J DiAngelo[3], C Jones[4], L Kadlec[5], J Kennell[6], SCS Key[7], J Leatherman[8], A Nagengast[9], L Reed[10], C Small[11], J Stamm[12], N Velaquez-Ulloa[13], M Wawersik[14], C Shaffer[15], W Leung[15], D Lopatto[16], SCR Elgin[15]. 1) Ohio Northern Univ; 2) Grand Valley State Univ; 3) Penn State Berks; 4) Moravian College; 5) Wilkes Uiv; 6) Vassar College; 7) North Carolina Central Univ; 8) Univ Northern Colorado; 9) Widener Univ; 10) Univ of Alabama-Tuskegee; 11) Medgar Evers College-CUNY; 12) Univ Evansville; 13) Lewis and Clark College; 14) College of William & Mary; 15) Washington Univ St. Louis; 16) Grinnell College.

The Genomics Education Partnership (GEP) is a consortium of faculty members from over 100 institutions who are involving students in Course-based Undergraduate Research Experiences (CUREs) in bioinformatics. As part of a comparative study on the evolution of the *Drosophila* Muller F element (dot chromosome), GEP students participate in sequence improvement and gene annotation of several *Drosophila* species. The student results are reconciled and pooled for final analysis; our paper on the expansion of the *Drosophila ananassae* F element will have 31 faculty and several hundred undergraduate co-authors. Recent curriculum development includes lessons to introduce eukaryotic gene structure to beginning students using a genome browser, and hands-on explorations of Hidden Markov Models and Dynamic Programming. Results from faculty and student surveys, quizzes, and focus groups indicate that CUREs are most effective, perhaps best when the faculty treat students like fellow scientists in a research group. Most of the student comments are positive, with students feeling that the effort to integrate learning with research experience is worthwhile. However, students do not show a change in “grit” in response to this experience. In collaboration with Galaxy, we are creating G-OnRamp, a suite of software and training materials that enables biologists with little IT training to create Genome Browsers with evidence tracks (*e.g.*, sequence similarity, gene predictions, RNA-Seq) for annotation of any eukaryotic genome. If interested in participating in a beta-users workshop for G-OnRamp, contact S. Elgin (selgin@wustl.edu). Supported by NSF IUSE #1431407, NIH R25GM119157, and Washington University in St. Louis.

722B Examining the Relationship between Perception of the Flipped Learning Environment and Performance in an Undergraduate Genetics Course. *Judith Leatherman*[1], Nissa Yestness[2], Lacy Cleveland[2]. 1) School of Biological Sciences, University of Northern Colorado, Greeley, CO; 2) MAST Institute, University of Northern Colorado.

Education literature clearly demonstrates the importance of active learning strategies for student success in undergraduate STEM courses. It is less clear, however, which type of active learning is most effective at promoting student learning. One educational innovation that is receiving much attention currently is the Flipped Classroom, where students learn the didactic material outside of class, often in the form of online lectures, then the class time is used for application and discussion of the material. In this study, we compared undergraduate Genetics courses that both had active learning components: one (non-flipped course) had about 80% lecture with 20% of the class period used for active learning, while the other (the flipped course) had nearly 100% of the class time dedicated to active learning, and students watched videos of the material before class.

Comparison of exam scores between the flipped and non-flipped courses showed no significant differences (exams 1-5, respectively, $p = 0.30$, $p = 0.39$, $p = 0.39$; $p = 0.37$, and $p = 0.17$). Thus, changing the *amount* of active learning

did not improve student exam performance. We also used a survey to collect data on students' perceptions of the flipped learning environment. Survey results revealed that 43%, 16%, and 41% of the students were satisfied, neutral towards, or dissatisfied, respectively with the flipped learning environment. We found that students' perceptions of the flipped learning environment were not correlated in any way to their performance on exams ($\chi^2 = 6.560$, df = 6, p = 0.374). Finally, survey data indicated that instructors implementing a flipped classroom should 1) plan their in-class activities to ensure time for students to complete the activity and to receive feedback, 2) assist students with developing time-management strategies, and 3) use a predictable format (e.g. assignments due on the same day every week).

723C Immersion Science Program: Inclusion of High School Students in Novel Laboratory Research *Alana O'Reilly*[1,2], Dara Ruiz-Whalen[2], Eric Lee[1,2]. 1) Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA; 2) Immersion Science Program, Fox Chase Cancer Center, Philadelphia, PA.

The development of all living organisms depends on a balanced diet. 75 years ago, nutritional requirements of *Drosophila melanogaster* were defined, resulting in development of a chemically defined culture medium that has been used to mechanistically connect genetic mutations and developmental defects without influence of dietary variation. The power of this system has not been used for the converse, however, to determine how nutrients influence developmental signal transduction. Recently, we defined a novel signaling role for dietary cholesterol in the *Drosophila* ovary. Association of cholesterol with its receptor triggers a kinase-dependent signaling cascade that controls levels of Hh available to epithelial stem cells. Thus, cholesterol consumption regulates the balance between Hh sequestration and release, allowing for rapid responses to changes in nutrient status and providing a mechanism for controlling egg production rates based on nutrient availability. Based on this initial study, we propose that a balanced diet is defined as the sum total of dietary compounds necessary to trigger responses in multiple cell types required for organ function, with each nutrient eliciting a specific signaling response. To create a large scale and low cost method for achieving testing this idea, we developed a laboratory research training program for high school students called the Immersion Science Program (ISP). Students first learn boot-camp lab techniques in the context of a reverse genetic screen to uncover specific dietary compounds that elicit phenotypic responses linked with specific developmental signal transduction pathways. Based on screening results, students develop self-designed hypotheses and experimental plans to determine the cellular and molecular consequences of nutrient treatment on flies bearing mutations in signaling effectors linked with observed phenotypes. To date, 80 students have completed our in-house program, and we recently extended the approach to high school classrooms in the Philadelphia region, resulting in two scientific publications since 2013. Our approach is dually powerful: in the short term, we will genetically map targets of dietary supplements using a comprehensive developmental genetics approach, while providing hundreds of students each year with the skills necessary to succeed in STEM majors. Our long term goal is to uncover new general principles governing the structural and dynamic effects of dietary compounds on protein function *in vivo*.

Full Late Abstracts

724A Defining Canoe/Afadin's role in apical-basal polarity establishment in early *Drosophila* development *Kia Perez-Vale*[1], Teresa Bonello[2], Mark Peifer[2,3]. 1) Curriculum in Genetics and Molecular Biology; 2) Department of Biology; 3) Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3280, USA.

During embryonic development, adult homeostasis, and wound healing, cells need to change shape and move while maintaining tissue integrity. Cell-cell adherens junctions (AJs) are essential for cell adhesion and cell shape changes. They also serve as key polarity landmarks, defining the boundary between apical and basolateral domains. Loss of cell-cell junctions and polarity are crucial for cancer metastasis. Thus, it is critical to understand the mechanism of action of junctional and polarity proteins, which allow them to accomplish these roles. An outstanding model to study apical-basal polarity establishment is the cellularizing *Drosophila* embryo, where 6000 cells form and polarize in 30 minutes. Previous work from our lab revealed that Canoe (Cno; homolog of human Afadin) is acting at the top of the hierarchy by regulating the polarized localization of Bazooka (Baz; homolog of human Par3), which in turn positions proteins of the cell-cell AJ apically. The first question that I set out to address is where within the cell Cno functions, and how it localizes there. Cno's structure suggests a scaffolding role, allowing it to link AJ proteins and actin. We are particularly interested in the two N-terminal Ras association (RA) domains, for which the small GTPase Rap1 is the preferred binding partner. Rap1 is thought to activate Cno, but the mechanism by which it does so remains unclear. Cno localizes to AJs in a Rap1 and actin-dependent manner. Surprisingly, we found that Cno still localizes to AJs after removal of the Rap1 binding-domains (CnoΔRA::GFP), in a manner similar to exogenous full-length Cno (CnoFL::GFP). Based on these results we hypothesized that either Rap1-binding activates Cno from an auto-inhibitory conformation or that endogenous wild-type Cno facilitates CnoΔRA recruitment to AJs. To test the first hypothesis, which predicts that CnoΔRA should be active independent of Rap1, I knocked down Rap1 in

CnoΔRA::GFP or CnoFL::GFP backgrounds. This prevented localization of both forms of GFP-tagged Cno to AJs, suggesting Rap1 binding is unlikely to simply relieve autoinhibition. I also determined that endogenous Cno does not facilitates AJ localization of CnoΔRA::GFP, suggesting that Rap1 acts in a more indirect way to facilitate Cno localization. Parallel work in the lab is exploring how Rap1 is activated and how active Rap1 regulates Cno localization. Together, these data will reveal the mechanisms driving the most upstream steps in polarity establishment.

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725B Regulation of PP2A by STRIPAK mediates axonal transport of autophagosomes *Amanda L. Neisch*, Thomas P. Neufeld, Thomas S. Hays. Genetics, Cell biology and Development, University of Minnesota, Minneapolis, MN.

In neurons, autophagy has a crucial function in the clearance of misfolded proteins, protein aggregates, and damaged organelles to maintain cellular homeostasis and neuronal function. Autophagosomes form at distal sites in the axon and are transported along microtubules by the motor protein dynein to proximal sites of lysosomal fusion, resulting in the degradation of the encapsulated cellular material. In an RNAi screen carried out to identify proteins that regulate the attachment and transport of autophagosomes by dynein, we identified the Striatin family protein, Connector of kinase to AP-1 (CKA). We show that CKA functions within a larger multi-protein complex, Striatin-interacting phosphatase and kinase (STRIPAK), to regulate autophagosome transport. In addition, CKA co-immunoprecipitates with dynein and can directly bind to the autophagosomal membrane protein Atg8 in pull down assays *in vitro*. Using *in vivo* imaging, we show that the STRIPAK complex also regulates the transport of dense core vesicles, but not mitochondrial transport. CKA also co-transports with autophagosomes and dense core vesicles. These results suggest that the STRIPAK complex functions locally to regulate organelle transport. Within the STRIPAK complex, CKA functions as a regulatory subunit of the phosphatase PP2A. PP2A is required within the STRIPAK complex to mediate axonal transport, and we show that CKA-deficient, adult Drosophila exhibit PP2A-dependent locomotor defects. Together our work has shown that the STRIPAK complex provides phospho-regulatory activity that is important for regulating axonal transport of a subset of organelles. Our data suggest that CKA function within the STRIPAK complex is crucial to prevent axonal transport defects that contribute to neurodegeneration. One putative substrate of PP2A's activity appears to be CKA itself, as okadaic acid treatment leads to hyperphosphorylation of CKA. We are investigating the function of CKA phosphorylation in the regulation of axonal transport. Currently, we are also attempting to identify additional substrates of the STRIPAK complex's PP2A activity and further define the STRIPAK complex found within neurons using BioID proximity labeling and mass spectrometry.

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726C Spatial organization of ER exit sites by Tango1 controls export capacity *Min Liu*, Feng Zhi, Hongmei Ke, Ying Liu, Tianhui Sun, Jianli Dai, Wenhong Cui, Jose C. Pastor-Pareja. School of Life Science, Tsinghua University, Beijing, China.

A key event in the secretion process is the exit of cargo from the endoplasmic reticulum (ER) towards the Golgi apparatus. Exit of cargo from the ER takes place at specialized ER domains called ER exit sites (ERES), where COPII vesicles assemble. In mammalian cells, loss of Tango1 and other related proteins of the MIA/cTAGE family present at ERES has been shown to impair secretion of very large secretory cargo such as Collagens and ApoB-containing lipoprotein particles. Using SIM superresolution microscopy and analysis of Tango1 mutant phenotypes, we found that *Drosophila* Tango1, the only MIA/cTAGE family member in fruit flies, is a critical organizer of the ERES-Golgi interface. Tango1 rings at ERES cups hold COPII carriers and Golgi in close proximity at their center. Loss of Tango1, present at ERES in all tissues, reduces ERES size and causes ERES-Golgi uncoupling, which impairs secretion of not just Collagen, but also of all other cargoes we examined. We additionally found that Tango1 can both self–interact and interact with GTPase Rab1 and cis-Golgi protein GM130. Further supporting an organizing role of Tango1, its overexpression creates more and larger ERES. Our results indicate that spatial coordination of ERES, carrier and Golgi elements through Tango1's multiple interactions increases general secretory capacity in *Drosophila* and allows secretion of large cargo.

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727A *SCF^{evl}* regulates Dpp signaling during *Drosophila* development *Nannan Zhu*[1], Wen Dui[1], Renjie Jiao[1,2]. 1) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; 2) Sino-French Hoffmann Institute, Guangzhou Medical Universiy,Guangzhou,China.

The conserved *Decapentaplegic*(Dpp) pathway plays important roles in controlling cell proliferation and cell fate determination. Deregulation of the Dpp pathway has been implicated in several types of cancers. Previous studies have demonstrated that post-translational modifications, such as phosphorylation, of the Dpp signaling components are involved in regulating the signaling activity. Ubiquitination, a covalent binding of an ubiquitin group to substrate proteins, mediated by E3 ligases, has been reported to play a role in Dpp pathway. However, whether ubiquitination mediated specifically by the SCF (Skp1-Cul1-F-box) family of E3 ligase is involved in the Dpp pathway remains unknown.

The SCF E3 ligase is implicated in many signaling pathways and consists of multiple subunits, in which F-box determines the specificity of substrates. Our previous study showed that knockdown of several F-box genes resulted in phenotypes characteristic of Dpp pathway dysregulation. These findings suggest SCF E3 ligase may play a role in the Dpp pathway. Here, we conducted a genetic interaction assay between Dpp pathway components and candidate F-box genes and identified *evl* as a novel negative regulator of the Dpp pathway. Consistently, knockdown of *evl* led to an increase of Dpp target gene expression. We also generated an allele of *evl* through p-element mediated imprecise excision. *evl* mutants exhibited lethality at the second instar larval stage and *evl* mosaic flies showed an extra vein phenotype which resembled that of *evl* knockdown. Further mechanistic studies are in progress.

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728B In vivo evidence that Notch is activated by force exerted by Epsin-dependent ligand endocytosis *Paul Langridge*, Gary Struhl. Department of Genetics and Development, Columbia University, New York, NY.

Notch is a single pass transmembrane receptor that is activated by two proteolytic cleavages: the first (S2) occurs in the extracellular Negative Regulatory Region (NRR) and is required for the second (S3), which occurs in the transmembrane domain and releases the cytosolic domain, a transcription factor, for nuclear entry. S2 cleavage is induced by transmembrane ligands of the Delta/Serrate/Lag2 (DSL) family in a manner that requires ligand to be internalized in signal-sending cells by the endocytic adaptor Epsin. We have used a novel system of chimeric Notch and Delta that recapitulates DSL-ligand/Notch signaling and allows the strict control of the interaction of the ligand and receptor *in vivo*. These tools allow the dissection of the mechanism of Notch activation and here we focus on why the endocytic component Epsin is required for ligand to activate Notch. Our results suggest that Epsin recruits ligand to an endocytic pathway that is required to exert a mechanical force to activate the receptor.

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729C The Role of Aldolase in Receptor Tyrosine Kinase-driven Tumor. *Arunima Purkayastha*, Utpal Banerjee. University of California, Los Angeles, Los Angeles, CA.

The Receptor Tyrosine Kinase (RTK) pathway plays a key role in regulation of proliferation, differentiation and survival of cells. Constitutive or aberrant activations of components of this pathway leads to increased proliferation and metastasis. Recently we established a system where individual components of oncogenic signals and metabolic pathways can be readily elucidated, by inducing a glycolytic tumor in the *Drosophila* wing imaginal disc by activating the oncogene PDGF/VEGF-receptor (Pvr). We discovered that a single oncogene, Pvr (PDGF/VEGF-receptor) activation can trigger a sequential phosphorylation of the major kinases in ERK/MAPK, PI3K/Akt, and Src/JNK pathways to induce tumor formation. Additionally, this tumor formation was associated with a metabolic shift towards glycolysis (Warburg effect). By performing an RNA-seq experiment, we discovered that most of the glycolytic enzymes were upregulated in Pvr-activated discs (CW Wang et. al., Elife; 2016). We observed that Aldolase (Ald) was one of the most highly upregulated glycolytic enzyme. Interestingly, knockdown of Ald in the Pvr-activated background dramatically reduced the size of the tumor. This rescue of overproliferation was not observed by knocking down other glycolytic enzymes in the Pvr-activated background. We found that the knockdown of Ald was

associated with a concomitant decrease in the levels of F-actin in the imaginal discs. More importantly, we found that knockdown of Ald downregulated all the major signaling pathways, ERK/MAPK, PI3K/Akt, and Src/JNK, contributing to Pvr-induced overgrowth. Currently, we are investigating the mechanism by which Ald contributes to the regulation of Pvr-induced signaling pathways.

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730A Dscam4 is required for elimination of polarity-deficient cells in *Drosophila* *Lina ZHANG*[1,2], Ying Wang[1,2], Yang Yang[1,2], Yan Yan[1,2]. 1) Department of Life Science, HKUST, Hong Kong, Hong Kong; 2) Center for Systems Biology & Human Health, HKUST, Hong Kong.

Cell polarity complexes are evolutionarily conserved protein complexes essential for establishing and maintaining asymmetry in polarized cell types such as zygotes, epithelial cells and neural stem cells. The Scribble complex, a highly conserved cell polarity module, contains three scaffold proteins: Scribble (Scrib), Discs-Large (Dlg) and Lethal giant larvae (Lgl). These genes were discovered in *Drosophila* as "neoplastic tumor suppressor genes" (nTSGs). When imaginal disc cells are homozygous mutant for one of the *nTSG* genes, the imaginal discs grow into amorphous tumors that kill the larvae. Interestingly, when *nTSG* mutant cells are induced as mosaic clones and are surrounded by wild-type cells, the mutant cells are eliminated from eye and wing imaginal discs. How the growth outcomes of *nTSG* mutant cells are determined remains unclear.

Through transcriptome analysis, we identified *Down syndrome cell adhesion molecule 4 (Dscam4)* as a gene highly expressed in imaginal discs harboring *scribble* mutant clones in comparison with those bearing control clones. Genetic analysis demonstrates that Dscam4 is required for the elimination of *scribble* mutant cells in imaginal discs. DSCAM family proteins are known for their function in neural wiring and they are known to interact with other neuronal guidance molecules. We will present our study towards understanding how Dscam4 functions to promote *scribble* mutant cell elimination in epithelia.

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731B Functional investigation of a novel F-box protein during *Drosophila* muscle development *Honggang Wu*[1], Nannan Zhu[1], Renjie Jiao[1,2]. 1) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; 2) Sino-French Hoffmann Institute, Guangzhou Medical Universiy,Guangzhou,China.

SCF E3 ubiquitin ligases catalyze the covalent linking of the ubiquitin molecules onto substrates and play important roles in various biological processes. F-box proteins are a component of the SCF complex which specifically recognizes the substrates. Our previous screen in *Drosophila* investigated the requirement of F-box genes during development of multiple tissues by characterizing the phenotypes of genetic knock-down. To validate these results and perform further studies, we have generated alleles of an array of F-box genes via CRISPR-Cas9 mediated mutagenesis. We found that a presumptive null mutant of the gene *fbp5*, while both viable and fertile, exhibited semi-lethality around the time of eclosion, and escapers are severely defective in mobility. RNAi mediated knockdown of *fbp5* driven by muscle specific Gal4 also led to similar phenotypes, documenting a requirement of normal *fbp5* function for muscle development. We are employing genetic, histological and biochemical approaches to further characterize the defects caused by *fbp5* loss of function, identify genes and pathways affected and study the mechanism of *fbp5* in regulating muscle development and function.

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732C Genome sequence and transformation for the fly Sciara - a new/old model system that disobeys the rules for chromosome movement on spindles *Susan Gerbi*, John Urban, Yutaka Yamamoto, Jacob Bliss. Dept Molec, Cell Biol, Biochem, Brown Univ BioMed Division, Providence, RI.

The fly *Sciara* has long been recognized as an outstanding model system to elucidate questions of chromosome mechanics. *Sciara* offers many unique biological features, several of which impact chromosome movement on spindles:

• chromosome imprinting;• a monopolar spindle in male meiosis I; • non-disjunction of the X chromosome in male meiosis II; • chromosome elimination in early embryogenesis; • sex determination; evolution towards parthenogenesis; • germ line limited (L) chromosomes; • DNA amplification in salivary gland polytene chromosomes;

• high resistance to radiation.

We have now developed a toolbox to enable research on the unique features of *Sciara.* We have completed the *Sciara* genome with cutting edge approaches for assembly using long reads from the PacBio RSII and Oxford Nanopore MinION sequencing platforms, using Illumina reads for polishing, and using BioNano Irys optical maps for scaffolding. Genome annotation used RNA-seq data from the *Sciara* transcriptome interrogating both sexes at multiple stages. We have used the genomic data to identify sequences of "DNA puffs" that represent sites of DNA amplification in salivary gland polytene chromosomes regulated by ecdysone.

We have developed methods for transformation of *Sciara* to manipulate its genome. DNA has been introduced into ectopic sites in the *Sciara* genome using piggyBac. We present here a new method for site-specific integration of large DNA into the *Sciara* genome utilizing the preferred pathway in most cells of non-homologous end-joining (NHEJ). We have coupled NHEJ with obligate ligation-gated recombination (ObLiGaRe) for high efficiency precise insertion of large DNA into a unique double-strand break. This approach is easily applicable to a broad range of organisms, including those where a transformation system has not been available.

With the new toolbox of the genome assembly and transformation methodology, the time is now ripe to elucidate many canonical processes using the unique biological features of *Sciara*. We welcome new investigators [http://brown.edu/go/sciara-stocks]. **Consider adopting *Sciara* for your research programs!**

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733A An optogenetic tool for studying asymmetric cell division *Arnaud Monnard*[1,2], Clemens Cabernard[1]. 1) Department of Biology, University of Washington, Seattle, USA; 2) Biozentrum, University of Basel, Basel, Switzerland.

Asymmetric cell division (ACD) is an evolutionary conserved mechanism used by stem and progenitor cells to form differentiating progeny while maintaining a self-renewed stem/progenitor cell. Drosophila neuroblasts (NBs), the precursors of the fly nervous system provide a good model system to study ACD in the context of brain development.

It has been recently shown that two different pathways co-regulate ACD of NBs and particularly the formation and positioning of the cleavage furrow, the so-called spindle and polarity-dependent pathways. The polarity pathway regulates the timing of Myosin flow onset on the apical neuroblast cortex and the spindle-dependent pathway controls the relocalization of Myosin from the basal cortex. We hypothesize that the correct timing of Myosin flow onset helps creating physical asymmetry. We implemented an optogenetic approach and found that ectopic relocalization of the basal cell fate determinant Miranda is causing ectopic furrowing. We will use this approach to investigate the relationship between neuroblast polarity and Myosin dynamics in ACD.

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734B Investigating the role of putative regeneration genes of *Notophthalmus viridescens* using *Drosophila melanogaster* model. *A Mehta*[1], A Luz-Madrigal[2], PA Tsonis[1], A Singh[1]. 1) Department of Biology, University of Dayton, Dayton, OH, USA; 2) Department of Biology, Miami University , Oxford, USA.

Notophthalmus viridescens, Red-spotted newt, possess amazing capability to regenerate its organs including tail, limb, heart, brain, spinal cord, lens and other tissues. We have identified a novel family of proteins expressed in adult tissues during regeneration in newts by using a *de novo* assembly of the newt transcriptome that is combined with proteomic validation. Although, these proteins have no counterparts in public databases, they have a putative signal peptide suggesting the secretory nature of these proteins. To investigate the regeneration potential of these newt-specific genes (and given certain restrains with transgenic newts, such as time), we employed transgenic *Drosophila melanogaster* model to express these genes. We generated the transgenic flies containing candidate genes, and tried to evaluate their potential to rescue pattern defect mutants of *Drosophila melanogaster*. Simultaneously, we prepared samples for RNA sequencing to generate the snap shot of gene expression when the candidate genes are misexpressed. Using transgenic approach, these candidate genes were expressed in all the tissues of *Drosophila*, and samples for sequencing were collected at third instar larval (L3) stage. Interestingly, we have found that these genes are affecting regulatory machinery of *Drosophila melanogaster* by binding to the sequence specific sites in DNA and regulating the genes involved in Apoptosis and cell cycle. We are further evaluating the potential of these novel genes to rescue pattern defect mutants of *Drosophila melanogaster*. Our hypothesis is that if these genes are responsible for regeneration they will be able to rescue developmental cell death. The results generated from these studies will be presented in the conference.

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735C A novel Sd corepressor restricts tissue growth through antagonizing Yki/Sd activity *Yawen Guo*[1], Jiyong Liu[2], Qinghua Wu[1], Renjie Jiao[1,2]. 1) State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, CAS , Beijing, China; 2) Sino-French Hoffmann Institute, Guangzhou Medical Universiy, Guangzhou,China.

The transcriptional coactivator Yki occupies a pivotal position in the Hippo signaling pathway, and the expression of its target genes directly controls tissue growth. However, how Yki target genes are suppressed is poorly understood. Here we identify a novel Sd corepressor, SBP (Sd binding protein), and uncover its role in regulating Hippo signaling pathway. Unlike Yki, SBP negatively regulates the transcription of Yki target genes and thus restricts tissue growth. Our data show that SBP gain-of-function leads to small wings, and a reduction of Ex transcription; loss of SBP increases the transcription level of Yki target genes (*ex, bantam, DIAP1*) in both the wing discs and the follicle cells. Furthermore, we show that SBP overexpression suppresses the increased wing size caused by Yki overexpression, which suggests an opposite role between SBP and Yki in controlling organ size. In particular, we demonstrate that SBP forms a complex with Sd/Yki. In addition, we observe that SBP locates in the promoter region of *ex*. Taken together, we propose that SBP suppresses Yki target gene transcription through competing with Yki for Sd. Our findings shed light on the role of a novel Sd corepressor and establish a link between chromatin modification and tissue growth.

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736A Control of cellular proliferation by the Salmonella effector AvrA *Brian Robinson*[1], Liping Luo[1,2], Rheinallt Jones[1,2]. 1) Department Pathology and Lab Medicine, Emory University, Whitehead Bldg., 615 Michael St., Atlanta GA, 30322; 2) Department of Pediatrics, Emory University, Whitehead Bldg., 615 Michael St., Atlanta GA, 30322.

Certain enteric pathogens evade elimination by secreting proteins that have potent suppressive effects on innate immune responses. For example, the intracellular pathogen Salmonella secretes AvrA which specifically inhibits host JNK pathway signaling. We created a transgenic Drosophila harboring avrA, allowing directed expression of AvrA in fly tissues. We previously showed AvrA is a potent inhibitor of pro-apoptotic JNK signaling in Drosophila. Here, we report that AvrA expression in a background of constitutive Eiger expression resulted in increased proliferation of Drosophila epithelial tissue and importantly of Drosophila hemocytes, whereas AvrA activity present in the absence of Eiger did not. Evaluation of downstream signaling pathways reveals cell autonomous proliferation and activation of several pathways, including elevated ERK-signaling. Together, these studies show Salmonella has evolved a protein that inhibits the pro-apoptotic JNK pathway and also stimulates epithelial tissue and phagocyte growth under pro-inflammatory conditions.

737B The Effect of Coconut Oil-Induced Stress Conditions on *Drosophila* Bahviors and Viability *Sandra Leal*, Dynesha Powell. Mathematics and Natural Sciences, Harris-Stowe State University, Saint Louis, MO.

We previously determined that pharmacologically-induced oxidative stress and genetic mutations of alleles within several major stress pathways both recover the *midline* adult mutant bristle phenotype characterized by a 50% loss of eye bristle (Chen et al., 2015; University of Southern Mississippi). Each eye bristle of about 450 are generated from a neuronal sensory organ precursor (SOP) cell lineage undergoing a series of asymmetric divisions to create terminal daughter cells of the bristle complex. By continuing these studies with undergraduate researchers at Harris-Stowe State University (Saint Louis, MO), we serendipitously discovered that adult wild-type flies exposed to solid media containing 30% coconut oil at 25°C died within 3-5 minutes. By observing oil-treated flies under the microscope, it appeared they were not drowning in or sticking to the media although we cannot rule out asphyxiation as the cause of death. We are now repeating studies to: 1) Gather enough data for statistical analyses, 2) Assay the time- and dosage-sensitivity of the coconut oil treatment on male and female adult flies, and 3) Examine the effects of other essential oils on adult male and female fly viability and whether there is an age-dependent effect. We are then preparing to transition to a mosquito model system for comparative analyses towards developing improved and safer alternatives to N, N-diethyl-meta-toluamide (DEET) as well as natural Eucalyptus oil treatments in the event mosquitos develop resistance to current repellents in use. Given the influx of mosquito populations into the United State carrying a variety of dangerous viruses, it is imperative to continue developing safe and effective repellents for future use.

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738C Effects of diet on genetically obese Drosophila *Allen G. Gibbs*[1], Elias P. Jacobs[2], Alyssa A. Caplan[3], Deja Y. Clay[4], Shemuel T. Patton[5]. 1) University of Nevada, Las Vegas NV; 2) SUNY-Fredonia, Fredonia NY; 3) Vassar College, Poughkeepsie NY; 4) Fort Valley State University, Fort Valley GA; 5) Nevada State College, Henderson NV.

Over 90 generations of selection for starvation resistance in outbred populations of *Drosophila* have resulted in flies that are extremely obese, even when fed a standard *Drosophila* diet. Obesity can also be induced in normally lean flies by rearing them on a low-protein, high-sugar diet. We therefore investigated whether starvation-selected flies respond differently to dietary changes than controls. Have starvation-selected populations reached their maximum lipid content, or can they be made even more obese through dietary manipulations? We reared starvation-selected and unselected lean control larvae on diets containing 5 yeast:sucrose ratios, ranging from 90% yeast:10% sugar (Atkins diet) to 10% yeast:90% sugar (American diet), as well as 3 different caloric concentrations. Starvation-selected and control flies had similar responses to rearing diet: development to adulthood was delayed on high sugar diets, and flies reared on high sugar diets eclosed with significantly greater lipid stores. Total protein levels were not affected, indicating that these flies were fatter, not simply larger due to extended larval development. To determine whether lack of micronutrients contained in yeast could have affected these results, we reared flies on high-sugar media containing vitamins, trace metals, cholesterol or RNA, as well as a combination of all of these. Lipid content did not differ from that of flies reared on un-supplemented media. We conclude that starvation-selected flies have not become as obese as possible, despite long-term directional selection favoring lipid storage. Supported by IOS-1355210 and DBI REU 1358896 from NSF and R15-GM100395 from NIGMS.

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739A Parental and Larval Diet Restriction Influences Longevity and Gene Expression Transgenerationally *Jason W Tresser*. Biological Sciences, Biola University, La Mirada, CA.

Caloric restriction is known to promote longevity in various species presumably through modulations in various metabolic pathways. In fruit files, diet restriction (DR) via lower yeast content in food has been shown to significantly extend lifespan. Microarray studies in *Drosophila* indicate that DR also modulates epigenetic factors, suggesting the possibility that offspring from diet restricted flies may inherit a propensity for long life through genetic imprinting. We report here that offspring from flies subjected to DR have differences in developmental timing, longevity and the expression of genes involved in metabolism and chromatin modification. We are investigating if these differences are amplified in a population of flies reared on DR for multiple generations.

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740B 4E-BP is a target of the GCN2-ATF4 pathway during Drosophila development and aging. *Min-Ji Kang*[1], Deepika Vasudevan[2], Kyunggon Kim[1], Hyung Don Ryoo[2]. 1) Dept of Biomedical Sciences, University of Ulsan College of Medicine, Seoul, South Korea; 2) Dept of Cell Biology, New York University School of Medicine, New York, NY 10016.

Reduced amino acid availability attenuates mRNA translation in cells and helps to extend lifespan in model organisms. The amino acid deprivation-activated kinase GCN2 mediates this response in part by phosphorylating eIF2α. In addition, the cap-dependent translational inhibitor 4E-BP is transcriptionally induced to extend lifespan in Drosophila melanogaster, but through an unclear mechanism. Here, we show that GCN2 and its downstream transcription factor, ATF4, mediate 4E-BP induction, and GCN2 is required for lifespan extension in response to dietary restriction of amino acids. The 4E-BP intron contains ATF4-binding sites that not only respond to stress but also show inherent ATF4 activity during normal development. Analysis of the newly synthesized proteome through metabolic labeling combined with click chemistry shows that certain stress-responsive proteins are resistant to inhibition by 4E-BP, and gcn2 mutant flies have reduced levels of stress-responsive protein synthesis. These results indicate that GCN2 and ATF4 are important regulators of 4E-BP transcription during normal development and aging.

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741C Dietary Restriction modulates sleep patterns in *Drosophila Melanogaster* *Behzad Varamini*, Lindsey Braden, Sierra Corban, Hayley Joel, Liz Katema. Biola University, La Mirada, CA.

Sleep deprivation has catastrophic metabolic and physiological consequences in numerous organisms, from fruit flies to humans. Dietary restriction (DR), a limitation of calories in the absence of malnutrition, has been shown to dramatically improve the health and longevity of a number of species, specifically overcoming some of the molecular and neurological deficits seen in aging and sleep decline. Using a 17-beam infrared-light paradigm, this study demonstrates that restriction of amino acids has significant effects on sleep/wake patterns in *Drosophila Melanogaster*. Further work will examine the genes activated by and necessary to achieve this effect.

742A BK_{Ca} (Slo) channel regulates mitochondrial structure and function, and lifespan in Drosophila *Shubha Gururaja Rao*[1], Piotr Bednarczyk[2], Atif Towheed[3], Kajol Shah[1], Priyanka Karekar[1], Beverly Reyes[1], Elisabeth Van Bockstaele[1], Adam Szewczyk[2], Douglas Wallace[3], Harpreet Singh[1]. 1) Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA; 2) Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw 02-093, Poland; 3) Center for Mitochondrial and Epigenomic Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA 19104.

Large conductance calcium and voltage-activated potassium channels (BK_{Ca}/Slo), are known for their roles in neuronal excitability, neurotransmitter release, hormone secretion and circadian rhythm in Drosophila as well as vertebrates. Originally discovered in Drosophila as *slowpoke* (*slo*), BK_{Ca} localizes to plasma membranes of various eukaryotic cells. Recently, BK_{Ca} has also been placed in mitochondria of neuronal and murine adult cardiomyocytes. Pharmacological and genetic approaches showed that BK_{Ca} plays an active role in neuroprotection and cardioprotection. However, their direct role in regulating mitochondrial structure and function are not completely elucidated. Using Drosophila *slo* mutants, we have discovered that ablation of *slo* results in structural and functional disintegration of mitochondria. The absence of *slo* resulted in an increase in electron transport chain activity, ATP-production, and reactive oxygen species generation and accumulation in cells. We further localized Slo to the inner membrane of mitochondria of Drosophila. Since mitochondria play a direct role in determining the longevity, we tested the lifespan of *slo* mutants and found that absence of *slo* inexplicably reduced the lifespan of Drosophila. We hypothesized that increase in mitochondrial ROS is affecting the life span of flies and tested it by feeding flies with paraquat, a ROS generator. As expected, paraquat feeding enhances the mortality of *slo* flies. Our study establishes a novel physiological role of *slo* in regulating the life span of Drosophila by regulating mitochondrial structure and function.

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743B Brain insulin cells sense cold via Gustatory Receptor 28b for the control of diapause in *Drosophila* *Yusuke Hara*, Noriyuki Ojima, Sae Yaoita, Hiroki Ito, Daisuke Yamamoto. Dept of Dev Biol & Neurosci, Grad Sch of Life Sci, Tohoku Univ, Sendai, Miyagi, Japan.

Diapause is a metabolic state in which animals minimize energy consumption and increase stress resistance to overcome unfavorable environmental conditions. We induce reproductive diapause in *Drosophila melanogaster* by exposing newly emerged female flies to low temperature, starvation, and short-day photoperiod. We show that diapause is promoted by silencing whereas suppressed by activating the brain insulin-like peptide producing cells (IPCs), which are responsive to light and temperature; under non-diapausing conditions, low temperature induces depolarization with spikes, whereas under diapausing conditions IPCs acquire tolerance to cold, barely generating spikes. We further demonstrate that knock-down of Gustatory Receptor 28b in IPCs abrogate cellular cold responses and inhibit to enter diapause. We propose that an exposure to sustained environmental stress switches the excitation state of IPCs from active to quiescent, which drives a fly to enter diapause.

744C X-ray crystallography and computational molecular dynamics of *Drosophila* striated muscle myosin II isoforms predict a basis for isoform-specific properties *James Caldwell*[1], Dan Mermelstein[2], Ross Walker[2], Girish Melkani[1], Tom Huxford[1], Sanford Bernstein[1]. 1) San Diego State University, San Diego, CA, USA; 2) University of California, San Diego, CA, USA.

Drosophila melanogaster contains one gene, *Mhc*, encoding all striated muscle myosin II isoforms. To gain insight into how alternative exon selection imparts myosin protein isoform biochemical and biophysical specificity, we employed X-ray crystallography and computational molecular dynamics. His-tagged recombinant proteins encoding

an indirect flight muscle myosin isoform (IFI) or an embryonic body wall myosin isoform (EMB) were expressed in and purified from the indirect flight muscles (IFM) of engineered fly lines lacking endogenous IFM myosin. The purified myosins retain ATPase activity similar to that of their corresponding untagged isoforms. Myosin subfragment-1 (S1) containing each myosin heavy chain motor domain and the essential light chain was crystallized and we determined the three-dimensional structure of IFI S1 at 2.5 Å resolution and EMB S1 at 2.2 Å resolution (PDB 4QBD). They are the first insect myosin protein structures determined by X-ray crystallography. The enzymatic state for both structures is post rigor, as determined by comparison with known myosin structures. For EMB, two copies of the myosin molecule with slight conformational differences were resolved in the asymmetric unit. The electron density revealed a citrate molecule (contained in the crystallization condition) in the nucleotide-binding pocket. For IFI, there is one myosin molecule in the asymmetric unit, with ADP in the nucleotide-binding pocket. A 500 nanosecond molecular dynamics simulation was run using the GPU accelerated AMBER 14 software suite and the FF14SB force field on the EMB crystal structure as well as on a model of IFI created by introducing the alternative amino acid sequences into the EMB structure. Analysis of the dynamics data included calculating the root mean square fluctuations. This resulted in the identification of two regions in IFI with significantly higher mobility than EMB: one contained the C-terminal portion of the relay domain and the other was the converter domain. The differences in conformation within regions of the proteins encoded for by alternative exons suggest a source of the observed physiological differences in the embryonic and adult flight muscle fibers. (Funded by NIH R01GM32443 to SIB)

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745A Characterizing a role for Dreadlocks, Misshapen, and the Arp2/3 complex in growth and stability of the germline ring canals Olivia Crowe, Ashley Kline, Marina Tipold, *Lindsay Lewellyn*. Biological Sciences, Butler University, Indianapolis, IN.

The fly egg develops from a multicellular structure called an egg chamber. Over the course of several days, the egg chamber progresses through 14 distinct stages before giving rise to a mature egg. The egg chamber contains a central cluster of 16 germ cells (1 oocyte and 15 supporting nurse cells) that is surrounded by a layer of somatic epithelial cells. Within the germ cell cluster, intercellular bridges called ring canals allow the transfer of materials from the nurse cells to the developing oocyte. The structural integrity and growth of these intercellular bridges is essential for fertility. We have identified two novel ring canal proteins, the SH2/SH3 adaptor protein, Dreadlocks (Dock), and the Ste20 family kinase, Misshapen (Msn). Both Msn and Dock localize to the ring canals, and depletion of either protein by RNAi leads to defects in ring canal size and structure. Strong depletion of Misshapen using the maternal triple driver (MTD-GAL4) led to a significant decrease in ring canal diameter and frequent collapse. This phenotype was similar to that observed upon depletion of the Arp2/3 subunit, ArpC2. In contrast, depletion of Msn using the nanos-GAL4 driver led to over-expansion of the ring canals compared to controls; this phenotype was similar to that observed upon depletion of Dock or ArpC2 using this driver. Dock and Msn genetically interact in other developmental contexts, and both have been linked to the Arp2/3 complex. The Msn homolog, NIK, phosphorylates and activates the Arp2/3 complex, and Dock was shown to genetically and biochemically interact with the Arp2/3 activator, SCAR, during myoblast fusion. Therefore, we are currently testing the model that Msn and Dock cooperate to promote Arp2/3 activity and ring canal growth. Future work will explore genetic and localization dependencies between Dock, Msn, and the Arp2/3 complex.

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746B Wingless promotes EGF signaling in the Follicle Stem Cell to maintain self renewal. *Rebecca Kim*, Todd Nystul. Anatomy, UCSF, San Francisco, CA.

The adult stem cell niche microenvironment is essential for maintenance and self renewal of the cell within that environment, ensuring a consistent population of stem cells available for regeneration of differentiated daughter cells. In the Drosophila ovary, follicle stem cells (FSCs) produce and maintain the epithelium of the eventual oocytes. These FSCs reside at the anterior edge of the tissue where they regularly divide throughout oogenesis. Like many epithelial tissues, the FSCs require the Wnt/Wingless and EGF signaling pathways for self-renewal. Abrogation of either pathway leads to FSC loss whereas constitutive activation of either pathway inhibits daughter cell differentiation. These findings demonstrate that precise activation of Wingless and EGF signaling within the narrow range of the FSC niche is essential to preserve stem cell identity while allowing daughter cells to differentiate. However, it remains unclear how this specificity is achieved, and how these signals cooperate to maintain FSC self renewal. We found that the specificity of Wingless signaling is not due to an inability of FSC

daughter cells to activate Wingless signaling or to cross-talk with the Hedgehog or Notch pathways. Instead, our data suggest that the Wingless ligand is locally delivered by nearby niche cells and spatially restricted to FSCs. We also find that FSCs with impaired Wingless signaling lack p-ERK, an indicator of EGF signaling, suggesting that Wingless signaling functions upstream of EGF signaling in a hierarchy of self-renewal pathways. We are currently investigating the mechanism by which Wingless signaling promotes EGF signaling and testing whether constitutively active EGF signaling can rescue the self-renewal loss caused by knocking out wingless signaling. These studies will contribute to our understanding of how epithelial stem cells are maintained both specifically and robustly, especially in a dynamic niche environment.

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747C "Survival of the fittest": Understanding the mechanisms by which follicle stem cell competition occurs in the niche *Sumitra Tatapudy*, Todd Nystul. University of California, San Francisco, San Francisco, CA.
The germarium, a structure at the tip of the ovariole of a *Drosophila* ovary, contains two follicle stem cells (FSCs) that are lost and replaced regularly by daughters of neighboring stem cells. However, some mutations can confer a competitive advantage or disadvantage to a mutant stem cell relative to the neighboring wild type stem cell. A genetic screen conducted in the Nystul lab through a collection of 126 mutants in essential genes on the X chromosome, identified candidate hypercompetitive and hypocompetitive alleles that increase and decrease stem cell replacement in the FSC niche respectively. Since hypercompetition mutations, by definition, enhance certain cellular features that are selected for by the competition process, understanding hypercompetition phenotypes will provide insight into underlying mechanisms that regulate niche competition. This study aims to elucidate mechanisms by which certain mutations confer a hypercompetition phenotype upon FSCs. By using FRT mediated mitotic recombination, we made stem cell mutant clones and measured the rate of stem cell replacement at six, twelve and eighteen days after clone induction. Our results confirm that *BenA*, an allele of an E2 Ubiquitin ligase *Bendless* and identified from the screen as an allele with the strongest putative hypercompetition phenotype, exhibited a significantly higher stem cell replacement rate as compared to the control. Given that *Bendless* plays a crucial role in cellular signalling, ongoing experiments are working towards identifying signaling pathways that are differentially regulated in *BenA* mutant and wild type stem cells. Additionally, using *BenA* and other candidate hypercompetitive alleles, we will investigate the role of differentiation, proliferation and apoptosis during stem cell replacement in the FSC niche.

Keywords: epithelium, stem cell, competition, germarium, follicle, signaling, differentiation, self renewal, ovary

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748A Molecular snapshot of a stem-cell like state *Manu D. Tiwari*[1,2], Andreas Wodarz[1,2]. 1) Cologne Cluster of Excellence in Cellular Stress Responses in Aging-associated Diseases (CECAD), Joseph-Stelzmann-Str. 26, 50937 Cologne, Germany, Cologne; 2) Anatomie I, AG Molekulare Zellbiologie, Geb. 37, Kerpener Str. 62, Uniklinik Köln, 50931 Cologne, Germany.
The precise division and differentiation of stem cells is essential for development of multicellular organisms. However, our understanding of the involved molecular factors and operative mechanisms is still fragmentary. To elucidate such factors and associated mechanisms, we performed paired-end RNA sequencing on FACS-isolated germline stem cell (GSC)- like cells from *Drosophila* ovaries. Due to the absence of *bag of marbles* differentiation factor, these mitotic cells fail to differentiate into cystoblasts. The gene expression profile of these cells differs in more than 2000 genes from that of normal germline cells. COMPLEAT and Cytoscape networking of these genes clusters them into several functional complexes involved in cellular homeostasis and fate determination including ribosomal biogenesis, mitosis, and transcription. We corroborate transcriptomics findings independently on a subset of these genes using functional genetics, compare the data with publicly available datasets, and postulate the mechanisms responsible for stemness and differentiation. Altogether, our data provide a snapshot of the molecular machinery operating in the maintenance and differentiaon of GSCs and serve as a resource for future investigations.

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749B Roles of *engrailed* and *bric-à-brac* in Germline Stem Cell Niche Formation in the *Drosophila melanogaster* Ovary. *Laurine Miscopein Saler*[1,2], Mathieu Bartoletti[5], Anne-Marie Pret[1,4], Laurent Theodore[3], Fabienne Chalvet[1,3,6], Sophie Netter[1,4,6]. 1) Institute of Integrative biology of the cell, CNRS - UMR 9198 - 91190 Gif Sur Yvette, France; 2) Paris-Saclay University, 91405 Orsay, France; 3) Paris-Sud 11 University, 91405 Orsay, France; 4) Versailles St Quentin University, 78035 Versailles, France; 5) Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912, USA; 6) equal contribution.

Stem cell recruitment and maintenance depends on the environment provided by a specific niche. The Germline Stem Cell (GSC) niche of the *Drosophila melanogaster* ovary is an excellent model to study niche formation : first, the signalling pathways involved in GSC maintenance are very well characterized, and secondly, cells composing the niche are easily distinguishable from the other somatic cells. In the adult ovary there are about twenty niches, each composed of a stack of 6-7 flattenened cells called a terminal filament (TF), associated cap cells (CCs) and escort cells (ECs). Each niche allows the maintenance of a small pool of 2-3 GSCs, and then the continuously production of oocytes throughout the lifetime of the female. Contrary to what is known about the maintenance of GSCs in niches in the adult , little is known concerning the molecular processes involved in the formation of functional niches. Niche formation takes place in the larval ovary in which TF cells (TFCs) are progressively flattened and stacked along a medio-lateral axis. During this process, TFCs are characterized by the expression of three transcription factors: Bric-à-brac1 (Bab1), Bric-à-brac2 (Bab2), and Engrailed. Results using a clonal loss of function approach indicate that Bab1 and Bab2 functions are necessary for the flattening and stacking of TFCs, as well as for *engrailed* expression in these cells. We also demonstrate, using a clonal gain of function approach, that Bab1 and Bab2 are not sufficient for induction of flattening and stacking of ectopic somatic cells, nor for ectopic *engrailed* expression in the larval ovary. However, induction of clones expressing all three genes, *bab1*, *bab2* and *engrailed,* ectopically is sufficient, under certain conditions, for ectopic recruitment of GSCs in the pupal and adult ovary. Therefore, our results suggest that Bab1, Bab2 and Engrailed act synergically in GSC niche formation.

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750C Wingless and neuronal activity drive cortactin expression to allow synaptic plasticity at the *Drosophila* neuromuscular junction *Marizabeth Perez*[1], Carihann Dominicci[2], Carolina Maldonado[2], Bruno Marie[2]. 1) University of Puerto Rico, Rio Piedras Campus, Institute of Neurobiology-201 Blvd del Valle, San Juan, PR 00901; 2) University of Puerto Rico, Medical Science Campus, Department of Anatomy and Neurobiology, Institute of Neurobiology-201 Blvd. del Valle, San Juan, PR 00901.

Major signaling molecules such as Wingless (Wg) are essential for the plasticity of the nervous system. Recently, it was shown that the *Drosophila* neuromuscular junction (NMJ) serves as a model to understand the molecular mechanisms underlying activity-dependent synaptic plasticity. Upon repeated stimulations, the NMJ shows formation of de-novo synaptic structures which are dependent on the Wg signaling pathway. Still, little is known about the cellular changes controlled by Wg that allow plasticity.

Here we focus on the actin regulator Cortactin (Cttn). Using genetics and confocal microscopy, we assess the role of Cttn in regulating activity-dependent synaptic plasticity. We show that Cttn is present at the NMJ pre- and post-synaptically and that de-novo synaptic structures related to synaptic plasticity are dependent on pre-synaptic Cttn. Indeed, these synaptic structures that appear after high neuronal activity contain Cttn at an early stage of their formation. In addition, we show that Cttn protein levels increase after repeated stimulations and that this increase is required for activity-dependent plasticity. To strengthen these results, we blocked action potentials and neurotransmitter release with the use of paralytic (para) and synaptotagmin (syt) mutants respectively. After repeated stimulations, there is no increase of Cttn and no plasticity in para and syt mutants, thus neuronal activity is required for the increase of Cttn and synaptic plasticity. Lastly, since the Wg pathway is required for activity-dependent synaptic plasticity, we tested wether the increase in Cttn after stimulation is also dependent on this pathway. To do so, we asked whether Cttn intensity was affected in Wg mutant larvae and frizzled (fz2) RNAi transgenic larvae (Fz2 is the Wg pre-synaptic receptor). We found that Cttn is not increased after stimulation in both Wg and fz2 deficient larvae and that plasticity is also impaired. This suggests that the pre-synaptic Wg signaling is required for the increase of Cttn and for plasticity. Overall our results strongly suggest that during repeated stimulation the expression of Cttn is required for the regulation of synaptic plasticity under the control of Wg signaling.

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751A Neuronal gluconeogenesis regulates glucose homeostasis via neuropeptide signaling in the *Drosophila* brain *Tetsuya Miyamoto*, Hubert Amrein. Molecular & Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

Gluconeogenesis, the synthesis of sugars from simple carbon molecules is essential to maintain sugar homeostasis. Interestingly, the key gluconeogenic gene, *Glucose-6-phosphatase* (*G6P*), is exclusively expressed in the central nervous system in *Drosophila melanogaster*. This is in marked contrasts to vertebrates where gluconeogenesis is essentially restricted to the liver and kidney. Here we explore the function of gluconeogenesis in the fly brain.

The GAL4/UAS system revealed that *G6P* and *Fructose-1,6-bisphosphatase* (*fbp*), two gluconeogenic genes, are co-expressed in several brain neurons including the two dorsomedial neuropeptide F (NPF) expressing neurons. Using a fluorescent glucose sensor, we demonstrate that application of alanine to *ex vivo* brain preparations significantly increased intracellular glucose levels in NPF neurons, providing physiological evidence for gluconeogenesis. Neuronal gluconeogenesis was confirmed *in vivo*, which showed that NPF neurons had higher glucose levels than other, non-gluconeogenic neurons in a *G6P* dependent manner. Taken together, these results provide physiological evidence for gluconeogenesis in the fly brain.

Given the highly restricted expression pattern of *G6Pase* to NPF neurons, we hypothesize that gluconeogenesis is employed as a novel mechanism for the regulation of neuropeptide signaling. To test this idea, the effect of gluconeogenesis on several aspects of neuronal physiology was assessed. We found that gluconeogenesis promotes the intracellular transport of NPF from the cell body to axon terminals without changing neuronal activities.

G6P mutant flies developed hypoglycemia during starvation, a phenotype that was rescued by a *G6P* transgene expressed in the *G6P-GAL4* expressing neurons. Importantly, TrpA1-mediated activation of *G6P-GAL4* expressing neurons also rescued the phenotype, suggesting that neuronal gluconeogenesis is functionally equivalent to neuronal activation.

Together, our studies suggest that a specific subset of brain neurons exploit gluconeogenesis to facilitate neuropeptide signaling to prevent hypoglycemia.

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752B The role of actin in axon regrowth following pruning *Shiri Yaniv*, Hagar Meltzer, Idan Alyagor, Oren Schuldiner. Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

How neurons determine their internal growth state during development and following injury is not well understood. Developmental neuronal remodeling, an evolutionarily conserved process used for wiring nervous systems of vertebrates and invertebrates, requires neurons to switch between different growth states, including initial outgrowth, pruning and regrowth. An attractive model that allows the genetic exploration of these growth states during development is the stereotypical remodeling of the *Drosophila* mushroom body (MB) γ neurons. During metamorphosis, MB γ neurons first prune their larval dendrites and axons and then regrow new, adult specific, connections. We have previously found that the formation of a stable nuclear receptors complex comprised of UNF (nr2e3) and E75 (nr1d1) promotes regrowth via the TOR pathway. Surprisingly, mutations in the UNF/E75/TOR pathway specifically affect developmental regrowth but not initial axon outgrowth of MB neurons, indicating that these distinct processes have unique underlying molecular mechanisms.

In order to further explore the mechanisms that control developmental regrowth we performed RNAseq of brains dissected at different developmental times. One group of genes that are upregulated prior to regrowth are genes that regulate actin dynamics. This includes Chickadee (Chic, the fly profilin) known to bind G-actin and increase the elongation rate of actin filaments and has already been implicated in regrowth. By performing a genetic screen we found that Enabled (Ena), a protein that stimulates F-actin assembly and elongation, is specifically required for axon regrowth but not for initial axon growth. Similarly, we found that Chic is also specifically required for developmental regrowth. Structure function analysis of Ena suggests that the interaction between Ena and Chic is required for Ena function, but overexpressing Chic can circumvent the requirement of Ena for regrowth. We are currently expanding our efforts to uncover the mechanisms by which Ena and profilin regulate axon regrowth. Furthermore, we are studying actin dynamics by using several F-actin binding reporters in WT and in mutant neurons. Taken together, our work should increase our understanding of actin dynamics during axon growth.

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753C Parallel Hormonal Pathways in Social Context-dependent Memories of *Drosophila*

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While it is commonly accepted that long-term memory (LTM) formation follows short-term memory (STM), recent studies have revealed parallel neural processes for each. We provide evidence that hormonal state regulates STM and LTM through apparently distinct pathways in *Drosophila*. We used the courtship conditioning paradigm for memory, whereby courting males experiencing rejection by mated females subsequently court receptive virgin females less avidly. We show that the ecdysis triggering hormone (ETH)-juvenile hormone (JH)-dopamine (DA) axis is essential for STM recall in courtship-conditioned males during an early adult critical period. With regard to LTM, both ETH and JH signaling also are required, but unlike STM, both hormonal signals appear to have direct actions on target neurons. Increased ETH release from Inka cells enhances memory through *de novo* protein synthesis, whereas increased JH release from but corpora allata does not, indicating that ETH directly affects the nervous system for LTM. Unlike STM, the JH-DA circuit is not required for LTM. We also found that both ETH receptor and JH receptor Met are required for LTM formation. Expression of ETH receptors in the male brain suggests that a subset of memory consolidation circuits may be regulated by ETH. This study provides new insight into the role of hormonal state in social context-dependent behavioral plasticity.

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754A The role of *yellow* in male mating success of *Drosophila melanogaster*

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Drosophila melanogaster exhibits sexually dimorphic courtship behavior; while males perform an elaborate courtship ritual, females do not (Demir & Dickson 2005). Sex-specific isoforms of transcription factors Doublesex (Dsx) and Fruitless (Fru) have been implicated in specifying male courtship behavior (Villella & Hall 1996; Demir & Dickson 2005; Rideout et al. 2010). However, the identities and roles of their downstream factors remain largely unknown. Previous reports that loss-of-function mutants of a pigmentation gene *yellow* show abnormal courtship behavior and decreased mating success suggest that *yellow* may be a potential downstream gene of Dsx or Fru (Bastock 1956; Drapeau et al. 2003; Drapeau 2005). Data from yeast one-hybrid assay and ChIP sequencing suggest that Dsx binds to *yellow*, leading to the hypothesis that *yellow* functions downstream of Dsx (Clough et al. 2014; Kalay et al. 2016). This study tests the hypothesis that *yellow* expression in *dsx* cells affects mating success in male *D. melanogaster*. The GAL4/UAS system, which drives expression of a gene downstream of UAS in cells expressing GAL4 transcription factor from its upstream cell-specific promoter, was used to selectively knockdown or overexpress *yellow* in *dsx* cells. Male flies that had *yellow* expression knocked down in *dsx* cells mated less successfully than control males, indicating that *yellow* in *dsx* cells is necessary for wild-type levels of male mating success. Male flies of *yellow* loss-of-function mutant background overexpressing *yellow* in *dsx* cells mated more successfully than control males, suggesting that *yellow* in *dsx* cells is sufficient for wild-type levels of male mating success. Overall, these data suggest that *yellow* expression in *dsx* cells is important for the regulation of male mating success of *D. melanogaster*.

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755B Dopamine Receptor (Dop1R1) is required in the Mushroom Body for Regulation of Daytime Sleep

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Sleep is an essential behavioral state of rest that is regulated by homeostatic drives to ensure a balance of sleep and activity, as well as independent arousal mechanisms in the central brain. Dopamine has been identified as a critical regulator of both sleep behavior and arousal. Here we present results of a genetic screen that selectively restored the Dopamine Receptor (*DopR/Dop1R1/dumb*) to specific neuroanatomical regions of the adult *Drosophila* brain to assess requirements for DopR in sleep behavior. We have identified subsets of the mushroom body that utilize DopR in daytime sleep regulation. These data are supported by multiple examples of spatially restricted genetic rescue data in discrete circuits of the mushroom body, as well as immunohistochemistry that corroborates the localization of DopR protein within mushroom body circuits. Independent loss of function data using an inducible RNAi construct in the same specific circuits also supports a requirement for DopR in daytime

sleep. Additional circuit activation of discrete DopR$^+$ mushroom body neurons also suggests roles for these subpopulations in sleep behavior. These conclusions support a new separable function for DopR in daytime sleep regulation within the mushroom body. This daytime regulation is independent of the known role of DopR in nighttime sleep, which is regulated within the Fan Shaped Body. This study provides new neuroanatomical loci for exploration of dopaminergic sleep functions in *Drosophila*, and expands our understanding of sleep regulation during the day versus night.

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756C Fly Stampede 2.0: A next generation optomotor assay for walking behavior in *Drosophila melanogaster* *K. Tellez*[1], S. Kim[2], G. Buchan[1], T. Lebestky[1]. 1) Biology, Williams College, Williamstown, MA; 2) Dana Farber Cancer Institute, Boston, MA.

Optomotor behavior represents a stereotyped locomotor response to visual motion that is found in both vertebrate and invertebrate models. The Fly Stampede assay was developed to study an optomotor response in freely walking populations of *Drosophila*. Here we share optimized assay designs and software for production of a modified stampede assay that can be used for genetic screens, and improved tracking outputs for understanding behavioral parameters of visual-motion responses and arousal state of individual animals. Arousal state influences behavioral performance in the stampede assay. As proof of principle experiments we show parametric modulation of visual stimuli and startle stimuli in both wildtype and mutant flies for the type I family dopamine receptor Dop1R1 (DopR). *DopR* mutants are hyperactive and perform poorly in the stampede assay, suggesting a potential role in visual perception and/or arousal. The stampede assay creates an efficient platform for rapid screening of mutant animals or circuit manipulations for investigating attentional processes in *Drosophila*.

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757A RNA as an attractive tastant and helps in rapid growth in larval *Drosophila* *Dushyant Mishra*[1], Natasha Thorne[2], Hubert Amrein[1]. 1) Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX; 2) National Institute of Health Bethesda, MD.

Rapid growth characterizes the larval stage of *Drosophila*: From time of hatching until puparium formation, larvae double their weight about every 10 hours by increasing their weight from about 1 microgram to 1 milligram. To accommodate this rapid growth, larvae eat almost constantly, and hence, they need to evaluate their food with regard to nutritious content. We have previously shown that *Gr43a* encodes the main larval sugar receptor, and that this receptor is used in peripheral taste neurons to sense dietary sucrose and fructose, as well in neurons in the brain to evaluate circulating hemolymph fructose.

Here, we report that larvae also show strong appetitive behavior towards ribose and ribose containing biomolecules, including inosine, uridine and RNA. We find that the members of the small subfamily of conserved Gr28 proteins are expressed in taste neurons, and that Δ*Gr28* mutant larvae fail to sense ribose or ribose-containing substrates. Moreover, a genomic rescue construct including all six *Gr28* genes, or individual *Gr28* genes expressed in taste and central neurons under the control of *Gr28a-GAL4* completely rescue ribose preference, indicating that Gr28 proteins are necessary for ribose sensing. Using the Ca^{2+} sensor CaMPARI, we show that Gr28a expressing taste neurons respond strongly to ribose containing chemicals such as RNA, inosine, and uridine, as well as ribose itself, but not to fructose and sucrose. In addition, by using a chemically defined (holidic) diet, we show that inosine and uridine are essential for timely larval growth and survival, and that these nucleotides can be replaced with RNA, but not ribose or DNA, without any detrimental effect on growth and survival. These findings indicate that *Drosophila* larvae exploit the presence of ribose to sense RNA or RNA metabolites in their food to sustain rapid larval growth. RNA is abundantly found in fruit pulp, as well as in yeast, the major protein source of growing larvae. To our knowledge, our finding is the first report of a taste receptor that senses a large biomolecule.

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758B Embryonic pioneers of the *Drosophila* brain central complex *Ingrid V. Andrade*[1], Jaison Omoto[1], Bao Nguyen[1], Satkartar Khalsa[1], Volker Hartenstein[1], Albert Cardona[2], Nadia Riebli[3]. 1) Molecular, Cellular and

Developmental Biology, UCLA, Los Angeles, CA; 2) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn VA 20147; 3) Biozentrum, University of Basel, Basel Switzerland.

The compartments and nerve connections of the central complex (CX) are the only parts of the Drosophila brain that develop postembryonically during metamorphosis. We and others have identified the discrete neural lineages whose secondary (=postembryonically born) neurons form the CX. Among these are four type II lineages (DM1-4) that produce the majority of columnar neurons, cells interconnecting the different CX compartments in a topographically ordered way. We wondered what role, if any, the primary (embryonic) neurons of these lineages play during central complex development. We found a novel type of primary neurons with small, heterochromatin-rich nuclei and unbranched axons which pioneer the central complex already in the late embryo/early larva. Each DM lineage, as well as many other lineages, contains 5-8 of these "small undifferentiated (SU)" neurons; axons of the SU neurons of the DM lineages, which we call the "fan-shaped body pioneers" (FBps), form four bundles that are organized in a topographically ordered chiasmatic structure across the brain midline, the fan-shaped body primordium (prFB). Later differentiating secondary DM neurons imitate the precise order laid down by the FBps. We carried out ablation studies specifically affecting the FBps, and find significant changes in the pathways taken by secondary DM neurons.

759C The Molecular Basis Underlying the Association Between Gaucher Disease and Parkinson's Disease *Gali Maor*[1], Hermann steller[2], Daniel Segal[3,4], Mia Horowitz[1,4]. 1) Cell research and immunology, Tel Aviv University, Tel Aviv, 69978, Israel; 2) Strang Laboratory of Cancer Research, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA; 3) Department of Molecular Microbiology and Biotechnology Tel Aviv University, Tel Aviv, 69978, Israel; 4) Sagol Interdisciplinary School of Neurosciences, Tel Aviv University, Tel Aviv, 69978, Israel.

Gaucher disease (GD) is an autosomal recessive disease resulting from mutations in the β-glucocerebrosidase (GCase) encoding gene, *GBA*, which lead to accumulation of the GCase substrate glucosylceramide. GD patients and carriers of GD mutations have a significantly higher propensity to develop Parkinson disease (PD) in comparison to the non-GD population. This implies that the mutant GBA allele is a predisposing factor for development of PD in carriers of GD mutations. We have previously shown that in cells derived from patients of GD and carriers of GD mutations, mutant GCase molecules lead to ER stress and to activation of the ER stress response, known as the unfolded protein response (UPR). ER stress and UPR play a key function in development of PD.

We used the fruit fly *Drosophila melanogaster* to confirm that development of PD in carriers of GD mutations results from the presence of mutant GCase. We have generated a *Drosophila* model for carriers of GD mutations by using transgenic flies expressing the human N370S, L444P or 84GG mutant GCase variants. We could recapitulate UPR activation in the flies carrying the different GCase mutants. Moreover, flies expressing the transgenic mutant human GCase variants in their dopaminergic cells developed Parkinsonian signs. They presented death of dopaminergic cells and had a decreased locomotion. They also had a significantly shorter life span. ER stress and Parkinsonian signs could be rescued by growing the flies containing the N370S or the L444P mutant GCase variants in the presence of the pharmacological chaperone ambroxol, which binds and removes mutant GCase from the ER. In contrast flies expressing the 84GG mutant variant, which does not generate mature GCase, did not respond to the presence of ambroxol.

Our results strongly suggest that the presence of a mutant GBA allele in dopaminergic cells leads to ER stress and to their death, and contributes to development of Parkinson's disease.

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760A Study of The Effect of Spirulina (*Arthrospira platensis*) on Paraquat Sensitive Parkinson's Disease Model of *Drosophila melanogaster* *Komal R. Panchal*, Anand K. Tiwari. Genetics and Developmental Biology , Institute of Advanced Research, University, Gandhinagar-382007, Gujarat, India.

Study of the effect of Spirulina (*Arthrospira platensis*) on Paraquat Sensitive Parkinson's Disease Model of *Drosophila melanogaster*

Spirulina (*Arthrospira platensis*) is a cyanobacterium (blue-green alga) consumed by humans and other animals due to its nutritional value and pharmacological properties. Along with high protein content, it also contains high levels of antioxidant and anti-inflammatory compounds such as carotenoids, (β-carotene), phycocyanin and phycocyanobilin that indicates its possible pharmaco-therapeutic utility. In the present study using Parkinson's disease model in *Drosophila*, we have demonstrated the therapeutic effect of spirulina and its active component c-phycocyanin by performing survivorship and locomotor assay in Parkinson's disease flies. Our findings indicate that dietary supplementation of spirulina significantly improves the lifespan and locomotor activity of paraquat insulted

Parkinson's disease flies. Furthermore, supplementation of spirulina and c-phycocyanin individually and independently reduced cellular stress by deregulating the expression of cellular stress markers and significantly decrease in anti-oxidant enzymes activities in spirulina fed Parkinson's disease flies inclines to believe the involvement of antioxidant properties associated with spirulina in the modulation of stress-induced signaling in Parkinson's disease model of *Drosophila*. Our results suggest that antioxidant boosting properties of spirulina can be used as the nutritional supplement for improving lifespan and locomotor behavior in Parkinson's disease.

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761B The Role of Nutrient Sensors, CRTC and CREB, in Cardiomyopathy *Anjali Gupta*[1], Rolf Bodmer[1], Marc Montminy[2], Karen Ocorr[1]. 1) SBP Medical Discovery Institute, La Jolla, CA; 2) The Salk Institute, La Jolla, CA.

In liver and skeletal muscle, the CREB (cAMP responsive element binding protein) Regulated Transcriptional Co-activator (CRTC) has emerged as an important transcriptional co-activator involved in nutrient sensing. Although CRTC and CREB have been implicated in human metabolic diseases such as diabetes, their role in heart has not yet been studied. Calcineurin-mediated de-phosphorylation activates CRTC and enables its translocation into the nucleus. Because calcineurin is known to activate NFAT and subsequently hypertrophic cardiomyopathy, we hypothesize that CRTC signaling may play an important role in cardiomyopathy in parallel to the calcineurin/NFAT hypertrophic program.

The semi-intact fly heart model was used to explore the role of CRTC in the adult fly heart. We conducted loss- and gain-of-function studies to understand these effects on the fly heart phenotype. Heart performance indicators such as heart period, fractional shortening, stroke volume, and cardiac output were measured or derived from these studies. To determine the effects of CRTC knockout on the cellular structure of the fly heart tube, we used methods in immunohistochemistry. We stained the fly hearts with an antibody to pericardin (a collagen-IV like extracellular matrix protein) to determine fibrosis. We also examined the myofibrillar structure of the heart via actin staining.

Our studies indicate that CRTC is involved in maintenance of fly heart function and adult heart structure. CRTC knockout mutant fly hearts have significantly reduced diastolic and systolic diameters. These flies also show significantly reduced diastolic intervals resulting in reduced heart periods. These effects are rescued with CRTC overexpression in a CRTC mutant background fly. CRTC mutant flies show increased deposits of pericardin, an indicator of fibrosis, while actin staining indicates disorganized myofibrils. Finally, the CRTC mutant flies have reduced triglyceride levels compared to normal wildtype flies demonstrating a role for CRTC in metabolism. We have also conducted tissue specific RNAi knockdown of CRTC as well as tissue specific coverexpression of CRTC using the UAS-Gal4 expression system. These results indicate that CRTC effects are mostly heart autonomous. Additional data on these set of experiments will be presented.

In conclusion, we have identified a novel signaling pathway in the heart that may serve as a therapeutic target for cardiomyopathies resulting from metabolic disorders.

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762C *Drosophila CASK* mutants: a small genetic model of intellectual disability with microcephaly *Judith A Tello*[1], Dailu Chen[1], Wulfila Gronenberg[2], Linda L Restifo[1,2]. 1) Dept. of Neurology, University of Arizona, Tucson, AZ; 2) Neuroscience Graduate Interdisciplinary Program, University of Arizona, Tucson, AZ.

CASK encodes a highly conserved MAGUK-family scaffolding protein involved in brain development and function. Primarily found in neurons, CASK is localized to both pre- and post-synaptic zones, and also accumulates in the nucleus. Loss-of-function mutations in human *CASK* cause three X-linked intellectual disability disorders, of which microcephaly with pontine-cerebellar hypoplasia is the most severe. To model these disorders, we used the *Drosophila CASK* mutation, *∆18,* an imprecise-excision allele that eliminates full-length CASK protein, and its corresponding control, the precise-excision allele, *Ex33*. We analyzed brain size of *Δ18* homozygotes from serial sections of osmium-stained, plastic-embedded pharate-adult heads. Brain volumes were significantly reduced in both males and females. At least in males, head size was not reduced. Hence the phenotype is microencephaly (small brain). To investigate the cellular developmental basis of this defect, we examined neurite-arbor morphogenesis of larval CNS neurons in primary dissociated cultures. Visual inspection and quantitative analysis by NeuronMetrics™ revealed the "bushy" abnormality of size and shape in neurons lacking full-length CASK: small neurite arbors (reduced length, higher-order branches, and area) with increased branch density. Deletion mapping

confirmed that the phenotype comes from the *CASK* region. Heterozygotes (*Δ18/Ex33)* display an intermediate phenotype, indicating that bushy is semi-dominant. Neuronal expression of one transgenic copy of $CASK^{+}$ partially rescued the bushy phenotype of *Δ18* homozygotes. These data suggest that the small-brain phenotype caused by *CASK* mutations in flies and children results from defective formation of dendritic arbors and axonal projections, rather than from reduced neuron number. The Griffiths lab reported a locomotor deficit of adult *CASK* mutants. To better understand their spontaneous motor behavior, individual 1-day-old adults were recorded for 15-min intervals. *CASK*-mutant flies walked significantly less and stood for extended periods of time but, despite their apparent immobility, they groomed excessively. Handling and transfer to a novel environment transiently restored walking in mutant flies, indicating that basic neuromuscular function is intact. Drosophila *CASK* provides a powerful new model of human microcephaly, setting the stage for drug discovery.

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763A A new *Drosophila melanogaster* model reveals the influence of genetic variability on volatile anesthetic pharmacology Zachariah Olufs[1], Carin Loewen[2], Tyler Klobucher[3], Barry Ganetzky[2], David Wassarman[2], *Misha Perouansky*[1]. 1) Department of Anesthesiology, University of Wisconsin SMPH, Madison, WI; 2) Department of Genetics, University of Wisconsin, Madison, WI; 3) Mayo Medical School, Rochester, MN.

General anesthetics - a class of nonspecific drugs with pleiotropic effects – interact with a large number of molecular targets in multiple biological pathways. In addition to conventional phenotypes (immobility, hypnosis, and amnesia) and side-effects, anesthetics also have a wide spectrum of unconventional effects (acceleration of apoptosis, tissue protection from injury, and immunemodulation). However, with the exception of rare life-threatening conditions, limited information exists on the role of genetic variability in the quantitative expression of both conventional and unconventional phenotypes. Collections of wild type and mutant lines of *Drosophila melanogaste*r can serve to advance our understanding of anesthetic pharmacogenetics. To exploit the genetic diversity of flies, we developed a system to compare the pharmacodynamic and pharmacokinetic (PK/PD) properties of volatile anesthetics among multiple fly lines. We found that the comparative PK/PD properties of two volatile anesthetics (isoflurane and sevoflurane) observed in mammals are conserved in flies. Moreover, we found differences in anesthetic pharmacodynamics among fly strains from the Drosophila Genetic Reference Panel, which are derived from a naturally occurring population. Lastly, we found that flies carrying a mutation in Complex I of the mitochondrial electron transport chain ($ND23^{60114}$) are hypersensitive to volatile anesthetics, adding to prior findings in *Caenorhabditis elegans* (*GAS-1*) and *Mus musculus (Ndufs4)*. We conclude that *Drosophila melanogaster* is a suitable invertebrate model system to investigate the genetic determinants of conventional and unconventional anesthetic phenotypes, providing access to a sophisticated genetic toolbox at a moderate cost and with limited animal welfare concerns.

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764B Genomics of rapid adaptation on seasonal timescales in D. melanogaster *Sharon Greenblum*[1], Alan Bergland[3], Mary Catherine Berner[2], Subhash Rajpurohit[2], Susanne Tilk[1], Paul Schmidt[2], Dmitri Petrov[1]. 1) Biology Dept, Stanford University, Stanford, CA; 2) Biology Dept, University of Pennsylvania, Philadelphia, PA; 3) Biology Dept, University of Virginia, Charlottesville, VA.

While long-standing theory predicts that adaptation stems from infintesimal and incremental genomic change, large rapid phenotypic changes have been observed in wild and laboratory environments. Until now however, the tools to reveal the genomic signal underpinning such changes have been lacking. Here we present the results of a large-scale evolution experiment in Drosophila melanogaster, directly assaying the genome-level dynamics of adaptation on seasonal timescales. Our approach marks a turning point for studies of adaptation, combining the highly-replicated nature of laboratory experiments with the full range of natural selective forces, and yielding precise accounts of allelic trajectories within individual populations via deep sequencing across multiple timepoints. The novel use of large exposed outdoor cages both enables replication and prevents migration, affording crucial statistical power to downstream analysis. Using advanced statistical tools to confidently define genomic signatures of adaptation, we present our results in the context of phenotypic assays of these same populations, as well as prior observations of seasonal cycling of alleles in wild populations. These results stand as an initial real-time glimpse of the complex evolutionary dynamics of metazoan populations, and lay the groundwork for integrating theoretical and empirical accounts to truly understand how and why populations adapt.

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765C Parallel seasonal selection across *Drosophila melanogaster* populations *Heather Machado*[1], Alan Bergland[2], Paul Schmidt[3], Dmitri Petrov[1], Drosophila Rapid Evolution Consortium[4]. 1) Biology, Stanford University, Stanford, CA; 2) Biology, University of Virginia, Charlottesville, VA; 3) Biology, University of Pennsylvania, Philadelphia PA; 4) Drosophila Rapid Evolution Consortium.

Fluctuating selection can play a role in the maintenance of genetic variation. For species with several generations per year, the seasonal changes in climate and resource availability can result in fluctuating selection. We performed population genomic sequencing of 56 seasonal (spring/fall) samples collected from 19 North American and European *Drosophila melanogaster* populations. We find a significant enrichment of seasonally varying SNPs across populations. We find high concordance of allele frequency change across seasons and with latitude for SNPs that are both strongly seasonal and strongly latitudinal. However, we estimate that seasonal sites tend to be of relatively small effect size and that they are variable in their identity across populations, resulting in low predictability across populations and years. Nonetheless, we demonstrate that seasonal selection is a general phenomenon in *D. melanogaster*, implicating it in the maintenance of genetic variation.

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766A Genetics of behavioral and morphological evolution between *Drosophila elegans* and *D. gunungcola* *J. H. Massey*[1], D. L. Stern[2], P. J. Wittkopp[1]. 1) University of Michigan, Department of Ecology and Evolutionary Biology, Ann Arbor, MI; 2) Janelia Research Campus, Ashburn, VA.

Animal species often differ in ecologically important traits that involve changes in morphological structures and the behaviors that use them. Pigmentation and courtship behavior in *Drosophila* are remarkably diverse and genetically tractable traits that show patterns of correlated evolution between certain species. We mapped quantitative trait loci for a wing pigmentation trait and courtship behavior between *Drosophila elegans* and *D. gunungcola* to study the genetics of correlated phenotypic evolution. Future work will aim to use introgression-based mapping techniques coupled with CRISPR/Cas9 gene editing to identify the specific genes and mutations underlying these phenotypic differences.

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767B Birth of a Network *Gerid A Ollison*, Scott Roy, Blake Riggs. Molecular Biology, San Francisco State University, San Francisco, CA.

Splicing factors are proteins responsible for binding pre-mRNA transcripts, causing changes through the inclusion or exclusion of target exons. They cannot cannot function without an established splicing network. Splicing networks minimally require: 1) a functional splicing factor to affect expression of target genes, 2) tissue-specific expression of that functional splicing factor, 3) the presence of genes to be regulated with targeted exons, and 4) the sensitivity of targets to the regulatory molecule -a binding motif or splicing enhancer sequence.
LS2 is a retroduplicated paralog of the highly conserved ubiquitously expressed splicing factor U2AF50, and has evolved a distinct function in Drosophila. While it is clear how LS2 evolved from its progenitor U2AF50, how LS2 evolved a distinct function is largely unknown. To determine how LS2 evolved a distinct function, I will systematically reconstruct the evolutionary history of the LS2 regulated splicing network (1 through 4). Preliminary bioinformatic data suggests that the the functionality of LS2 arose in Scaptodrosophila, a sister species of Drosophila. To confirm this I will test functionality using molecular biology techniques. Furthermore, I am using genomic data to confirm orthologous features of LS2 target genes. While primary analyses using RNA-seq reads and Bowtie suggest LS2 has maintained testis specific expression throughout its existence, qPCR analyses will be employed to definitively confirm this assumption.
By studying each splicing network requirement independently, I how the LS2 promoted alternative splicing network evolved in Drosophila. The evolutionary history of this alternative splicing network will shed light on speciation and how lineages evolve novel genes and proteins with distinct function.

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768C Comparative genomics of *Drosophila melanogaster* using BioNano optical maps *Carrie Evans*, Evan Long, Emma Grooms, Joshua Udall. Plant and Wildlife Sciences, Brigham Young University, Provo, UT.

Drosophila melanogaster has been a model organism for genetic and genomic study. With the use of the BioNano Irys system, we are able to construct a physical genome map of multiple strains of *Drosophila melanogaster*. By fluorescently imaging nicked and labeled high molecular weight DNA, the images are assembled into a physical map. The physical maps were mapped to sequence-based maps to verify chromosome integrity and identify structural variations. This same method also allowed for the identification of regions of the genomes that may have been incorrectly assembled previously. These structural variants will be used to enhance our understanding of the evolutionary history of *Drosophila melanogaster* through the genome comparisons of BioNano and DNA sequence data.

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769A Inherently Probabilistic Invasion Dynamics of the Gut Microbiota Benjamin Obadia[1], Tuzun Guvener[1], Vivian Zhang[1], Javier Ceja-Navarro[2], Eoin Brodie[2], Bill Ja[3], *Will Ludington*[1]. 1) Molecular Cell Biology, UC Berkeley, Berkeley, CA; 2) Lawrence Berkeley National Lab, Berkeley, CA; 3) Scripps Research Institute, Jupiter, FL.

Background: The gut microbiome faces constant invasion by new colonizers, but only some successfully establish. We sought to understand underlying causes, specifically (i) probabilistic events, (ii) specific host and invader traits, and (iii) other members of the gut microbiota.
Methods: We examined these questions in the natural, low-diversity bacterial community of Drosophila melanogaster using gnotobiotic flies. We developed a modified capillary feeding system to individually dose bacteria to hundreds of flies and determine colonization. We measured spatial localization and also bacterial growth rate in the fly gut by developing a simple and widely applicable assay based on plasmid loss. We focused on three different strain variants of Lactobacillus plantarum (from human saliva, from Canton-S lab flies, and from wild flies).
Results: The surprising result is that some stable colonizers nevertheless reach a plateau in their dose-response of roughly 50% colonization, meaning that colonization is inherently probabilistic even for natural residents introduced at extremely high dose. We discovered anaerobic pockets and distinct spatial localization patterns. Overall our data suggest a bistable system where stochastic differences between growth and death rates in small populations induce transitions between colonized vs uncolonized. We derived a mathematical model to link population dynamics of small populations with quantitative dose-response theory. The model lets us calculate key parameters of establishment, and it indicates that limited gut habitat makes colonization probabilistic, but that many factors can cause habitat limitation, including strain physiology, host genetics, and other gut species.
Conclusions: Our data and model demonstrate hysteresis, whereby uncolonized guts stay uncolonized and colonized guts stay colonized, which could explain the long-term stability of individual human gut microbiota despite large differences between individuals. Our study suggests that gut colonization by commensals and pathogens alike may be inherently probabilistic, which has important implications for infectious disease and probiotic therapy.

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770B *Lucilia sericata* (Meigen) and *Chrysomya megacephala* (Fabricius (Diptera: Calliphoridae) development rate and its implications for forensic entomology *K. Verma*. Department of Genetics, Maharshi Dayanand University, Rohtak-124001, Haryana, India.

Forensic entomology is basically a science that is based on the scientific study of the invasion and succession pattern of arthropods with their developmental stages of different species found on the decomposed cadavers during legal investigations. The developmental rate of *Lucilia sericata* and *Chrysomya megacephala* was studied in beef liver for the correlation of time duration in each phase with the temperature and climate. The obtained data belong to *L. sericata* at temperatures between 22°C and 26°C (mean - 24°C) and relative humidity 50% ±10% and *C. megacephala* at temperatures between 23°C and 27°C (mean - 25°C) and relative humidity 55% ±10%. From the analysis of results, it was observed that in the climatic conditions of the study area, time since death assessment involving *L. sericata* was found to be with a potential of maximum 10–11 days and *C. megacephala* with 8–9 days. The data emerged as results from the present work would be beneficial for investigations involving decomposed dead body remains for the assessment of time since death.

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771C Nemo phosphorylates Prickle promoting its degradation and maintaining the Prickle/Spiny-legs isoform balance during planar cell polarity establishment in the eye *Giovanna Collu*[1], Andreas Jenny[2], Konstantin Gaengel[3], Ivana Mirkovic[1], Mei-Ling Chin[4], Ursula Weber[1], Michael Smith[1], Marek Mlodzik[1]. 1) Cell, 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, Bronx, New York, NY USA; 3) Uppsala University, Dept. Immunology, Genetics and Pathology, Rudbeck Laboratory C11, Dag Hammarskjölds Väg 20, 751 85 Uppsala, Sweden; 4) Transonic Asia Inc. 6F-3 No 5 Hangsiang Rd, Dayuan, Taoyuan County, 33747 Taiwan R.O.C.

Planar cell polarity (PCP) signaling is a highly conserved pathway that coordinates cell orientation and behavior across a wide range of tissues through directing cytoskeletal changes and providing cell fate input. Here we report that post-translational modification and degradation of the cytoplasmic PCP factor Prickle is required specifically in the R4 photoreceptor for correct development of the fly eye. The balance between the Prickle and Spiny-legs isoforms of *prickle* is critical to establishing correct PCP. We show that Nemo kinase (Nmo), formerly identified as a mediator only of the PCP-cytoskeleton axis, is also required to reinforce PCP establishment and cell fate input. Nmo genetically interacts with core PCP components, enhancing cell fate defects. Using mosaic analysis we show that Nmo is required in the R4 cell only during establishment of PCP in the eye. Nmo directly phosphorylates 8 sites within the Prickle C-terminus that are required to limit Prickle function. Loss of *nmo* function leads to increased Prickle protein levels in the eye disc. Genetic interactions point to a model in which dTak1-dependent Nmo phosphorylation of Prickle leads to Prickle degradation via the Cullin1/SkpA/Supernumery limbs complex. Thus Nmo is required in R4 during PCP establishment to serve as a check on excessive Prickle activity and maintain the *prickle* isoform balance.

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772A Fundamental Origins and Limits for Scaling a Maternal Morphogen Gradient *Chuanxian Wei*[1,2], Feng He[1], Honggang Wu[1,2], David Cheung[1], Renjie Jiao[2], Jun Ma[1]. 1) Division of Biomedical informatics, Cincinnati Children's Hospital Medical Center,Cincinnati, OH; 2) Institute of Biophysics, CAS, Beijing, China.

Tissue expansion and patterning are integral to development, but it is unknown quantitatively how a mother accumulates molecular resources to invest in the future of instructing robust embryonic patterning. Here we develop a model, Tissue Expansion-Modulated Maternal Morphogen Scaling (TEM^3S), to study scaled anterior-posterior (A-P) patterning in *Drosophila* embryos. Using both ovaries and embryos, we measure a core quantity of the model, the scaling power of the Bicoid (Bcd) morphogen gradient's amplitude n_A. We also evaluate directly model-derived predictions about Bcd gradient and patterning properties. Our results show that scaling of the Bcd gradient in the embryo originates from, and is constrained fundamentally by, a dynamic relationship between maternal tissue expansion and *bcd* gene copy number expansion in the ovary. This delicate connection between the two transitioning stages of a life cycle, stemming from a finite value of $n_A \approx 3$, underscores a key feature of developmental systems depicted by TEM^3S.

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773B Sol narae (Sona) is essential for the development of abdomen in *Drosophila.* *Ja-Young Kim*. Biological Science, KAIST, Daejoen, Daejoen, South Korea.

The fly abdominal epithelium is formed by finely regulated replacement of larva epithelial cell (LEC) with histoblasts, the anlagen of adult abdominal epithelial cells. Four histoblast nests are generated in each abdominal segment during the late embryonic stage, and are mitotically quiescent during the entire larval stage. After metamorphosis begins at early pupal stage, histoblasts go through rapid proliferation at the expense of pre-occupying LEC that are committed to apoptosis. This substitution process is tightly regulated to prevent the formation of any holes in the adult abdominal epithelium. Although general information on the expansion and fusion of histoblasts have been studied, the underlying mechanism of abdomen epithelium formation is still largely unknown.

Here, we introduce Sol narae(Sona) as a novel player in the formation of abdominal epithelium. Sona is a *Drosophila* ADAMTS (a disintegrin and metalloprotease with thrombospondin motif) that promotes Wg signaling. Sona is essential for larval tissue growth and development of flies. Loss of *sona* results in morphological defects such as small wing and kinked femur, and pupal lethality. Dead pupae of *sona* mutants have patches of undead LEC in abdominal epithelium, suggestive of lack of histoblasts. Knock-down of *sona* using histoblast *Gal4* caused large hole in abdominal epithelium and pupa lethality. We propose that Sona plays essential roles in *Drosophila* abdomen development.

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774C Tension-dependent vinculin dynamics at adherens junction during cell boundary oscillation *Y. Toyama*[1,2,3], Y. Hara[1], M. Shagirov[1]. 1) Mechanobiology Institute, Singapore; 2) Department of Biological Sciences, National University of Singapore, Singapore; 3) Temasek Life Sciences Laboratory, Singapore.

Throughout development, cell boundaries undergo contraction and elongation to change tissue morphology. In comparison to cell boundary contraction, which is mainly driven by Myosin II dependent contraction, the mechanisms of cell boundary elongation are still elusive. We used amnioserosa cells, which exhibit cell oscillation during *Drosophila* dorsal closure, as a model system to uncover the mechanics underling cell boundary elongation, and to elucidate how the junctional tension evolves during cell oscillation.

Here we show that the cell boundary elongation is predominantly driven by non-autonomous active process, which is associated with the transient flow of Myosin II in the neighboring cells pulling the vertices. Moreover, measurements of the junctional tensions at the specific phases of boundary oscillation by laser ablation, showed that the elongating boundaries exhibited lower tension in compare to the contracting boundaries. We extended our individual tension measurements to non-invasively estimate a tension map across the tissue. We further found that the vinculin, which is known to interact with adherens junction component a-catenin in tension dependent manner, accumulates to or dissociates from oscillating boundary, and the dynamics correlated with the estimated junctional tension. We propose that the level of vinculin at the cell boundary could be used to approximate junctional tension.

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775A Decoding the mechanism of sharp gene expression driven by an animal enhancer *Jeehae Park*, Gemma Johnson, Chiara Ricci-Tam, Anna Cha, Meghan Bragdon, Javier Estrada, Jeremy Gunawardena, Angela DePace. Systems Biology, Harvard Medical School, Boston, MA.

In animal development, sharp gene expression boundaries define specific cell fates. Developmental enhancers create sharp expression boundaries by integrating graded concentrations of input transcription factors to decide whether to turn their target gene on or off. Here we examine a canonical developmental enhancer in Drosophila embryos, hunchback P2 (Hb P2), which responds to the transcriptional activator Bicoid to create a sharp expression boundary at the midpoint of the anterior/posterior axis. Activity of the Hb P2 enhancer has long been thought to be controlled by cooperative binding of Bicoid to a cluster of high affinity and low affinity Bcd binding sites. We systematically interrogated cis and trans factors that influence the sharpness of Hb expression, and thus inform how Bcd acts cooperatively. We demonstrate that cis-mutations are not consistent with the classic model, and that factors other than Bcd, including other TFs and general cofactors such as Mediator, influence the sharpness and position of the Hb gene expression pattern. Our results thus support new explanations for sharp expression, perhaps based on higher-order cooperative interactions between TFs and co-factors, and/or dynamic processes.

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776B *small ovaries* regulates transposable element activity in the *Drosophila* ovary *Leif Benner*[1,2], Cale Whitworth[1,3], Kevin Cook[3], Brian Oliver[1,5], Dorothy A. Lerit[4,5]. 1) National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2) Department of Biology, Johns Hopkins University, Baltimore, MD; 3) Department of Biology, Indiana University, Bloomington, IN; 4) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA; 5) Co-corresponding Authors.

In the *Drosophila* ovary, excessive transposable element activity disrupts genomic stability resulting in somatic and germline cell death. piRNAs/PIWI proteins have evolved as a small RNA defense mechanism to repress the activity of these mobile genetic elements. This is achieved through direct cleavage of retro- and DNA-transposons as well as heterochromatic silencing at their genomic residence. The *small ovaries* (*sov*) locus was first identified as a set of non-complementing recessive female sterile alleles that resulted in a range of dystrophic ovary phenotypes. We performed deficiency mapping, duplication rescue, and whole genome DNA sequencing to determine that *sov* encodes *CG14438*. The *sov* locus encodes a predicted 371 kDa protein with 26 C_2H_2 zinc-finger domains. RNAi against *sov* in ovarian somatic tissues results in an extreme *sov* phenotype, while *sov* RNAi in the germline results in maternal-effect lethality during early embryogenesis without an overt *sov* phenotype. To determine the function of *sov,* we performed gene expression profiling on mutant and RNAi ovaries revealing an extensive upregulation of a myriad of transposons indicating that wildtype *sov* acts to repress transposon activity. The classes of transposons repressed by *sov* differ in somatic and germline cells. For example, the retrotransposons, particularly of the *gypsy* and *jockey* superfamily, are sensitive to *sov* in germ cells. While in the soma, most of the major classes of transposable elements show increased activity in the absence of *sov*. Recent work shows *sov* associates with HP1, along with repetitive transposable element sequences within this complex. We therefore conclude that *sov* has a role in transposable element regulation that is necessary for female germline viability and consistent with a piRNA/heterochromatin-associated response pathway.

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777C Single molecule dynamics of Bicoid in living Drosophila embryos revealed by lattice light sheet microscopy Mustafa Mir, Jenna Haines, Armando Reimer, Hernan Garcia, Michael Eisen, *Xavier Darzacq*. Genetics Genomics and Development, University of California, Berkeley, Berkeley, CA.

During early development in the Drosophila embryo, cell fates are determined over the course of just 2 hours with exquisite spatiotemporal precision. One of the key regulators of this process is the transcription factor Bicoid, the first known morphogen, which forms a concentration gradient across the long axis of the embryo. Bicoid’s primary role is concentration dependent transcriptional activation in the anterior of the embryo, and is generally thought to be non-functional at the posterior where there are only a few molecules per nucleus. Here we use lattice light sheet microscopy to overcome the technical barriers of sample thickness and auto-fluorescence, and for the first time visualize single molecules of Bicoid in a living embryo. We find that the off-rates of Bicoid binding to DNA are remarkably fast and do not vary across the embryo. The spatial distribution of binding events in anterior nuclei is isotropic as expected, however in the posterior nuclei we find that they are highly clustered and can enrich local concentrations to levels found in anterior nuclei. These results suggest a mechanism to locally modulate the on-rate of Bicoid binding in a gene specific manner and independently of the concentration gradient. This observation challenges the current model of the Bicoid morphogen model, since local concentration within the nucleus is regulated and suggests a previously unknown role of Bicoid dependent transcriptional regulation in the posterior Drosophila embryo.

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778A Investigation of eIF4E-BP Targets by TRIBE in Flies *HUA JIN*, Daxiang Na, Allegra Fieldsend, Weijin Xu, Michael Rosbash. Howard Hughes Medical Institute and National Center for Behavioral Genomics, Department of Biology, Brandeis University, Waltham, MA 02454, USA.

Our lab has developed a new technique, TRIBE (Targets of RNA Binding proteins Identified by Editing), to identify the targets of RNA binding proteins (RBPs) in small numbers of discrete cells (1). TRIBE employs a fusion of the catalytic domain of the RNA editing enzyme ADAR (ADARcd) to a RBP, the purpose of which is to identify its targets by high-throughput sequencing of bulk mRNA. RBP targets will be preferentially edited. Here, we successfully used TRIBE to determine eIF4E-BP (thor) mRNA targets in *Drosophila*. EIF4E-BP inhibits translation initiation through binding to 5’cap-binding protein eIF4E and repressing its activity. Although eIF4E-BP has been shown to be important in growth control, stress response, cancer, neuronal activity and circadian rhythms, it is unclear how it can preferentially repress a subset of mRNAs. We carried out eIF4E-BP-TRIBE in normal, serum starvation, and serum starvation with Rapamycin, conditions that activate eIF4E-BP. We found that eIF4E-BP is associated with specific mRNAs in S2 cells. Interestingly, a number of its targets correlate well with the known activity of eIF4E-BP, and GO term analysis indicates that targets are enriched in translation-related pathways. We provide the first evidence that eIF4E-BP associates with specific target mRNAs in cells, supporting the hypothesis that eIF4E-BP does not just

sequester eIF4E. Rather, eIF4E-BP must regulate some target mRNAs by interacting with them, perhaps as part of an eIF4E-eIF4EBP complex.

1. TRIBE: Hijacking an RNA-Editing Enzyme to Identify Cell-Specific Targets of RNA-Binding Proteins. McMahon, A, Rahman, R, Jin, H, Shen, JL, Fieldsend, A, Luo, W, and Rosbash, M. *Cell.* 2016 165, 742-753.

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779B Opposing RNA binding proteins LIN28 and FMR1 adjust the insulin sensitivity and expansion of stem cells required for adaptive intestinal resizing *Arthur Luhur*, Nick Sokol. Biology, Indiana University Bloomington, Bloomington, IN.

The adult Drosophila intestine grows or shrinks depending on the nutritional status of the animal. This adaptive resizing is driven by changes in the numbers of intestinal progenitor cells, which increase during nutrient availability, reach homeostasis, and decrease during nutritional scarcity. We previously found that the RNA binding protein LIN-28 is required for the diet-dependent expansion of intestinal progenitor cell population, but not their self-renewal. Because LIN-28 is abundant in stress granule-like puncta in the cytoplasm of progenitor cells, we screened known stress granule components for ones with similar expression patterns. From this analysis, we identified a second RNA binding protein, Fragile X Mental Retardation 1 (FMR1). Genetic analysis revealed that loss of *fmr1* enhanced the expansion of progenitor number, while overexpression of FMR1 reduced progenitor number. Since *lin-28* and *fmr1* loss-of-function mutations had opposite effects on progenitor number, we performed epistasis analysis and found that *lin-28* is epistatic to *fmr1*. Consistent with these results, we also found that *lin-28* and *fmr1* had opposite effects on the levels of an Insulin Receptor (InR) reporter as well as other insulin signaling activity markers (pAKT, tGPH) in progenitor cells. Furthermore, reduction of insulin signaling due to heterozygosity for *InR* or *chico* was sufficient to suppress the *fmr1* mutant progenitor number phenotype. Finally, we measured the insulin sensitivity of intestines using an *ex vivo* assay, and found that *fmr1* mutant intestines are more sensitive to lower levels of insulin than wildtype controls. Based on these results, we conclude that LIN28 and FMR1 form a post-transcriptional switch that adjusts the insulin sensitivity of progenitor cells and thereby regulates their division behavior. The current status of this work will be presented at the meeting.

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780C Mechanisms of Germ Plasm mRNA Localization *Whitby Eagle*, Elizabeth Gavis. Dept of Molecular Biology, Princeton University, Princeton, NJ.

Subcellular localization of mRNA is an effective strategy for establishing the asymmetric distributions of proteins necessary for developmental polarity. One process in which mRNA localization fulfills a critical role is specification and maintenance of germline fate during early development. By late stages of oogenesis, over 50 mRNA species are enriched within the germ plasm, the specialized cytoplasm at the posterior pole of the oocyte. Within the germ plasm, many of these mRNAs are co-packaged into complex ribonucleoprotein (RNP) granules known as polar granules, which facilitate their collective segregation into the primordial germ cells. This ensures inheritance of mRNAs that direct the production of proteins essential to germline development, viability, and function. Localization occurs during late oogenesis via diffusion and local entrapment, and requires specific recognition of cis-acting elements or ‘zipcodes’, often located in the 3’ untranslated region (UTR) of transcripts. However, these zipcodes, as well as the trans-acting protein factors that bind them remain largely uncharacterized.

We created a series of transgenic reporter constructs to define active zipcodes in the 3’UTRs of two functionally important germ plasm mRNAs, *pgc* and *gcl*. Using high resolution smFISH and quantitative image analysis we found that that the *gcl* 3’UTR contains multiple, distributed, partially redundant zipcodes. In contrast, we identified a discrete zipcode in the *pgc* 3’UTR. This zipcode proved responsible for ~40% of germ plasm transgene enrichment. However, it was insufficient to mediate localization except when coupled to additional sequences in the proximal portion of the 3’UTR. We therefore conclude that the initial polar granule docking interaction and subsequent transcript enrichment are likely to be functionally separable processes, regulated by different sequences in the 3’UTR. Additionally, we used an RNA affinity purification strategy coupled with mass spectrometry to identify proteins that bind preferentially to the *pgc* 3’UTR during late oogenesis. RNAi knockdown of several enriched candidates (Belle, Trailer Hitch, Zn72D, HnRNP-K, CG9684, CG6937) resulted in reduced mRNA localization to the germ plasm, consistent with a role for these proteins as localization factors.

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781A Investigating the establishment of nucleolar dominance during *Drosophila melanogaster* development *Natalie Warsinger-Pepe*[1,3], Yukiko Yamashita[2,3,4]. 1) Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Cell and Developmental Biology, University of Michigan, Ann Arbor, MI; 3) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 4) Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.

Nucleolar dominance is a previously identified phenomenon whereby particular ribosomal DNA (rDNA) loci are expressed and the others are silenced. Also, nucleolar dominance has been observed in the context of interspecific hybrids, where rDNA loci from one species are entirely silenced while those from the other species are dominantly expressed. The biological significance of nucleolar dominance remains a mystery.

In *Drosophila melanogaster,* the rDNA loci are located on the sex chromosomes. It has been previously shown in *D. melanogaster* males that the Y rDNA locus dominates over the X rDNA locus. In female *D. melanogaster,* both X rDNA loci are expressed, suggesting that no dominance occurs in wild type females. These previous studies were performed using condensed mitotic chromosomes from third instar larval neuroblast cells. The techniques previously used were limited to this specific cell type and cell stage, requiring condensed chromosomes to easily identify the X and the Y chromosomes by morphology. The inability to distinguish between X and Y chromosome rDNA transcripts limited these earlier studies from identifying when nucleolar dominance is established in other non-mitotic cell types. In this study, we use single-nucleotide polymorphism fluorescent *in situ* RNA hybridization (SNP *in situ*) to separately visualize X and Y rDNA transcripts in cell types with de-condensed chromosomes at various stages of *Drosophila* development. This study reveals that nucleolar dominance is not established in the developing embryo of male *D. melanogaster*; both X and Y rDNA transcripts are expressed in a majority of the cells in developing embryos throughout late-stage embryogenesis. Similar to previous studies, a majority of cells in male third instar larval brains express solely the Y rDNA locus. Female brain cells appear to express rDNA from both X rDNA loci, based on the presence of two nucleoli in many cells, although SNP *in situ* cannot distinguish transcripts from each separate X rDNA loci. Our results suggest that the establishment of nucleolar dominance is a developmentally programmed process, warranting further study for its biological significance.

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782B Automated Core Facility to Screen for Genetic Modifiers and Small Molecules in *Drosophila* *L. Li*[1,2], H. Chester[1,2], T. Onur[1,2], A. Perez[1,2], Y. Yao[1,2], M. Avalos[1,2], M. de Haro[1,2], I. Al-Ramahi[1,2], E. Do[1,2], J. Botas[1,2]. 1) Department of Human and Molecular Genetics,Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

We have established a core facility that includes state-of-the-art, custom robotic instrumentation to enable standardized, highly sensitive, and efficient quantification of motor impairments caused by neuronal / muscle dysfunction, aging or other causes. The high–throughput core facility is designed for genome-scale screens of genetic modifiers or small molecule screens.

The screening facility includes a number of integrated instruments that make it unique. A custom-made robotic instrument is designed for automated assessment of movement impairments. This central assay robot is fed by other pieces of automation designed to manipulate and transfer animals from vial to vial, sort embryos according to genotypes, and dispense small chemicals into *Drosophila* media.

The assay robot elicits a startle-induced negative geotaxis response by “tapping” flies housed in 96-vial arrays. Video cameras record and track fly movement, and the software deconvolutes the movement of each fly to compute more than two dozen individual and population metrics, including distance, speed, and stumbles. In our optimized protocol, cohorts of 15 flies are evaluated simultaneously in each vial, and the procedure is repeated ten times per replica at each time point. The automated, high-throughput system is capable of assaying 16 arrays (1,536 total vials) in ~4.5 hours.

We have used the instruments and software to successfully identify genetic modifiers and/or small chemicals ameliorating pathogenesis in *Drosophila* models of a variety of human disorders including Alzheimer’s disease (AD), Parkinson’s disease (PD) and Huntington’s disease (HD), spino-cerebellar ataxias (SCA1, 2, 7), myotonic dystrophy type 1 (DM1) etc.

This facility is now available to the research community. Please visit http://nri.texaschildrens.org/core-facilities/high-throughput-behavioral-screening-core.aspx.

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783C An individual fly-handling robot allows high-throughput longitudinal measurement of Drosophila social networks. *Tom Alisch*[1], William Long[2], Dave Zucker[4], Benjamin de Bivort[2,3]. 1) Department of Mathematics and Natural Sciences, University of Groningen, Groningen 9747 AG, The Netherlands; 2) Center for Brain Science and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, USA; 3) Rowland Institute at Harvard, Cambridge, Massachusetts 02142, USA; 4) FlySorterLLC, Seattle, Washington 98103, USA.

Drosophila melanogaster exhibits quantifiable non-random patterns during social interactions that can be summarized in a social interaction network (SIN). Parameters describing SINs, such as frequency and length of interactions, have been proposed to be under the influence of genetic variation and shown to remain stable within experimental trials (Schneider, Dickinson, & Levine, 2012). However, it is not yet established whether and to what extent SIN parameters on the group and individual level change over longer periods of time. Longitudinal SIN measurements are arduous and error-prone to perform by hand. For example, manually tracking individual flies' identity across time cumulatively increases chances for human error. To remedy this, we developed an individual fly-handling robot capable of autonomously performing high-throughput longitudinal experiments. Here, we demonstrate that the robot 1) autonomously sets up and performs multiple SIN analyses with dozens of fruit flies and 2) reliably tracks individual flies' identity across experiments.

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