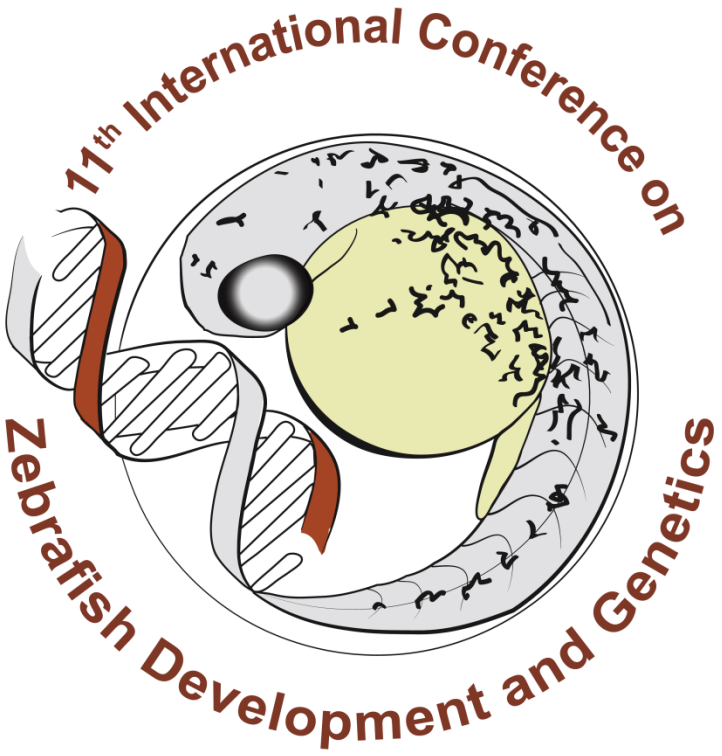

TEXT OF ABSTRACTS

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Abstracts

1. How Cells React to Noise: Direct Measurements Reveal Noise Attenuation Mechanisms in Retinoic Acid Signaling. ***Julian Sosnik***[1,2], *Likun Zheng*[3,2], *Enrico Gratton*[4,2], *Qing Nie*[3,2], *Thomas Schilling*[1,2]. 1) Developmental & Cell Biology, University of California, Irvine, Irvine, CA; 2) Center for Complex Biological Sciences, University of California, Irvine, Irvine, CA; 3) Mathematics, University of California, Irvine, Irvine, CA; 4) BME, University of California, Irvine, Irvine, CA.
The discovery that biological noise can generate diversity in genetically homogenous cell populations exposed to the same environmental cues, introduces the provoking possibility that noise is a tangible and important property of developing systems. Noise is ubiquitously present in developing organisms and stems from stochastic variations in gene transcripts, protein copy number, molecular interactions, protein activity, diffusion, etc. Genes expressed in developing rhombomeres in the embryonic zebrafish hindbrain arise with rough boundaries, which we have proposed sharpen, in part, due to noise-induced switches in gene expression. In this study we investigate the amplitude of noise in RA signaling, and how modulation of this variance affects the patterning of hindbrain boundaries. We have employed a new technique based on fluorescence lifetime imaging microscopy (FLIM) and phasor analysis to measure for the first time endogenous free intracellular concentrations of retinoic acid (RA) directly in vivo, and its variance both in space and time. Using this technique we show that noise is large in magnitude. We also show that cells use at least two molecular mechanisms to interpret their position within the morphogen gradient by regulating the average and variance of available RA in vivo. Cyp26a1, the main enzyme responsible for the degradation of RA, regulates mean levels of RA without altering the variance. In contrast, the cellular retinoic acid binding protein 2a (Crabp2a) regulates the variance (noise) in RA without altering the mean. These findings are among the first to show how cells regulate noise and open the possibility that regulation of noise has a biological function in the developing organism. We are currently focused on analyzing how manipulating noise in the RA signaling pathway affects the patterning of the developing hindbrain.

2. Ezh2 affects the maintenance of cellular identity during embryonic development of the zebrafish. ***Leonie M. Kamminga***[1,2], *Bilge San*[1], *Nadine Wittkopp*[3], *Federico Tessadori*[2], *Simon van Heeringen*[4], *Anne Lagendijk*[2], *Emily Noël*[2], *Jeroen Bakkers*[2], *René F. Ketting*[2,3]. 1) Molecular Biology, Radboud University Medical Centre Nijmegen, Nijmegen, the Netherlands; 2) Hubrecht Institute-KNAW and University Medical Centre Utrecht, Utrecht, the Netherlands; 3) Institute for Molecular Biology, Mainz, Germany; 4) Molecular Developmental Biology, Radboud University Nijmegen, Nijmegen, the Netherlands.
Polycomb group (PcG) proteins are transcriptional repressors and play a role in development, stem cell maintenance, and cancer. Knock-out mice for multiple PcG proteins show early embryonic lethality. We used the zebrafish to study the role of *enhancer of zeste homolog 2* (*ezh2*), a PcG gene responsible for setting the repressive H3K27me3 mark, during early development in further detail. We identified a mutant with a premature stop in *ezh2*. Homozygous mutants at first develop normally, but die around 12 days post fertilization (dpf) displaying a severe intestinal phenotype, in which tissue identity seems to be lost over time. We also generated embryos lacking both zygotic and maternal *ezh2*. Remarkably, these embryos survive gastrulation and die around 2 dpf with pleiotropic phenotypes in brain, heart, circulation, and the eyes. Moreover, the expression of *hox* genes, known targets of PcG, was shifted anteriorly. We used this system to study the effect of loss of maternal *ezh2* on gene expression. Strikingly, already before gastrulation there are major differences in gene expression between wildtype and maternal zygotic *ezh2* mutant embryos, while establishing a normal body plan. We then continued to analyze the observed heart phenotype in greater detail. This revealed striking developmental defects, accompanied with detachment of myocardial cells from the heart, a phenotype that is rarely observed. Marker gene analysis of these detaching cells suggests they may represent de-differentiated, or poorly differentiated cardiomyocytes. Our data show that Ezh2 is dispensable for early development and initial cell type specifications. Rather, Ezh2's primary role seems to be in maintenance of cellular identity at time points following tissue specification.

3. Imaging Endothelial Cell Dynamics during Vascular Network Formation. ***Heinz-Georg Belting***[1], *Loïc Sauteur*[1], *Anna Lenard*[1], *Alice Krudewig*[1], *Lukas Herwig*[1], *Nikolaus Ehrenfeuchter*[2], *Markus Affolter*[1]. 1) Dept. of Cell Biology, Biozentrum, Uni Basel, Basel, Switzerland; 2) Imaging Core Facility, Biozentrum, Uni Basel, Basel, Switzerland.
Vascular network formation can be subdivided into three processes - sprouting angiogenesis, anastomosis and regression -, which ensure growth, connectivity and efficient blood transport within a vascular tree. All these processes require dynamic cell behaviors, such as cell rearrangements, cell polarization, cell shape changes, which are driven to a large extent by endothelial cell-cell interactions. We are studying these cell interactions *in vivo* by high-resolution time-lapse analysis using fluorophore-tagged proteins. We find that blood vessel anastomosis follows a stereotypic series of events involving contact formation, apical-basal polarization followed by membrane invagination and cell rearrangements, which connect adjacent lumens and transform the initial unicellular tube into a multicellular tube, respectively. Analysis of blood vessel regression shows that this process follows the same principle as vessel anastomosis, however in reverse order. We have now analyzed the cell behaviors underlying sprout elongation. *In vivo* live-imaging shows that outgrowth of intersegmental sprouts underlies two different mechanisms: cell migration and cell elongation. Whereas the tip cells displays strong migratory behavior, extension of the stalk is mainly driven cell elongation. We find that loss of CDH5 function does not affect the migratory potential of tip cells. However, cell elongation of stalk cells is impaired. 3D-reconstruction and quantification of junctional interfaces reveal an inability of *cdh5*$^{-/-}$ stalk cells to transform the intercellular surface area from a round into an elliptical shape, suggesting incapacity to apply deforming forces onto endothelial cell junctions. In agreement with this view, we find that the cortical actin network is strongly disturbed in *cdh5*$^{-/-}$ stalk cells. Taken together, our findings support a cellular model for sprout elongation, in which CDH5 is specifically required in the stalk to promote cell shape changes by transmitting cytoskeletal forces, which deform intercellular surface areas and thus drive coordinated endothelial cell elongation.

Abstracts

4. *gsx1* specifies neurons that modulate the startle response. ***Sadie Bergeron***[1], *Nicole Carrier*[2], *Grace Li*[1], *Harold Burgess*[1]. 1) NIH, NICHD, PGD, Bethesda, MD, U.S.A. 20892; 2) U of Texas Health Science Center, Dept. of Pharmacology, San Antonio, TX, U.S.A. 78229.
Neuronal filtering mechanisms that normally prevent irrelevant sensory information overload to the brain are disrupted in neurodevelopmental disorders including schizophrenia. One such mechanism is prepulse inhibition (PPI) of the acoustic startle response (ASR). PPI suppresses the ASR and occurs when a non-startling stimulus precedes a startling stimulus by a short (< 1 s) interval. We sought to identify the neural circuitry that mediates PPI. To do this, we first generated transgenic zebrafish enhancer trap lines with CNS-specific expression of Gal4 by incorporating a tandem neuronal restrictive silencer element (NRSE) into our transgene. Neuronal specific lines were screened for PPI deficits by crossing them to a transgenic UAS-nitroreductase line allowing for spatial and temporally controlled ablation of neurons prior to behavior testing. Line *y252* has disrupted PPI, and we confirmed it is an enhancer trap for the transcription factor *gs homeobox 1* (*gsx1*). We found that brainstem *gsx1* neurons project to the region of VIIIth nerve sensory input to the Mauthner cell, and form synapses in this same region suggesting that they directly mediate PPI. Supporting this, acute optogenetic inhibition of *gsx1* neurons resulted in disrupted startle responsiveness. Surprisingly brainstem *gsx1* neurons are primarily glutamatergic despite their role in a functionally inhibitory pathway. Consistent with a role for glutamate signaling in PPI, treating larvae with an NMDA receptor antagonist reproduced the ablation phenotype. Intriguingly, the pattern of *Gsx1* expression is highly conserved during neural development in mice, therefore we analyzed *Gsx1* knockout mice for PPI to determine if our findings were relevant to mammals. We found reduced PPI in *Gsx1* knockout mice compared to wild type siblings. Gsx1 has an established role in forebrain interneuron development, thus these findings suggest that it may play a central role in the generation of neural circuits across multiple brain regions that are linked to altered sensory motor gating and neurological disorders.

5. A map of epithelial cell dynamics guiding morphogenesis of the zebrafish fin regeneration epidermis. ***Chen-Hui Chen***[1], *Stefano Di Talia*[2], *Kenneth Poss*[1]. 1) Department of Cell Biology and Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC; 2) Department of Cell Biology, Duke University Medical Center, Durham, NC.
Adult salamanders and teleost fish demonstrate a remarkable ability to regenerate amputated limbs or fins. Formation of a signaling epidermis is an early, defining event in appendage regeneration. Yet, little is known of the cell behaviors that create this key structure. Here, we generated a transgenic system to label epithelial cells on the surfaces of adult zebrafish fins with dozens of unique colors. In vivo imaging revealed that injuries are closed within minutes of fin amputation by sequential basal and suprabasal epithelial cell layer coverage, limiting loss of mesenchymal cells. Wound closure leads to extrusion of many epidermal cells at the amputation plane. Surprisingly, quantification of individual cell dynamics from live imaging of regeneration indicated that fin interray epithelial cells sharply change shape and mobility within minutes of amputation, whereas fin ray epithelial cells show a delayed response with limited shape changes. Blockade of stretch-activated channels inhibited closure by basal and suprabasal epithelial cells, while polarizing the differences in injury responses by interray and ray cell populations. Thus, by developing quantitative methods to assess epithelial cell morphology in live zebrafish tissues, we have revealed cell and tissue dynamics that underlie creation of the fin regeneration epidermis.

6. Mutation of zebrafish Kif5Aa tail domain causes axonal mitochondrial deficits in a mixed model of Hereditary Spastic Paraplegia/Charcot Marie Tooth Disease Type II. ***Philip D. Campbell***[1,3], *Kimberle Shen*[4], *Matthew Sapio*[2,3], *Thomas D. Glenn*[4], *William S. Talbot*[4], *Florence L. Marlow*[1,3]. 1) Developmental and Molecular Biology; 2) Molecular Pharmacology; 3) Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 4) Developmental Biology, Stanford University School of Medicine, Stanford, CA.
Defective axonal transport has been linked to neurodegenerative disorders and mutations in Kinesin motors (Kifs) implicated in axonal transport lead to central and peripheral nervous system defects. Human mutations in Kif5A, a member of the Kinesin-1 family, cause peripheral motor/sensory dysfunction classified as either Hereditary Spastic Paraplegia or Charcot Marie Tooth Type II (CMT2). However, the relevant cargo and basis of sensitivity of subsets of neurons remain unclear.
Here we show that homozygous loss of the C-terminal tail of Kif5Aa, but not Kif5Ab, causes a striking neurologic syndrome reminiscent of the human disorder, including motor spasticity, uncoordinated movements, increased seizure susceptibility, peripheral neuropathy, and partial blindness. Furthermore, both posterior lateral line and cutaneous nerve axons show clear signs of degeneration. Electron microscopy and transgenic approaches show a drastic reduction in axonal mitochondria, while presynaptic vesicles and lysosomes remain present. Mitochondria deficits are rescued cell autonomously by transient overexpression (OE) of wild-type Kif5Aa but not OE of Kif5Ab, Kif5Ba, or Kif5Bb, Kinesin-I family members thought to be functionally redundant with Kif5Aa. Thus, mitochondrial transport in peripheral sensory neurons specifically requires Kif5Aa.
To investigate functional redundancy with other Kifs, we tested whether *kif5Aa* genetically interacts with *kif1b*, another Kif implicated in CMT2 and mitochondrial transport. Compound *kif1b;kif5Aa* mutants display a striking absence of peripheral cutaneous innervation. Together, these results demonstrate that specific motors fulfill transport in discrete subsets of neurons, implicate defective mitochondrial transport as a key factor in human disease, and underscore the importance of evaluating functional redundancy between Kinesin motors.

7. Schwann cells and DCC direct regenerating motor axons onto their original path. ***Allison (Ali) F. Rosenberg***, *Roshan Jain, Clara Franzini-Armstrong, Michael Granato.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
A major challenge for regenerating peripheral axons is to cross the injury gap and navigate towards their original trajectory. Early during this process Schwann cell derived factors promote growth cone sprouting, yet whether Schwann cells only provide an adhesive substrate or actively direct axons towards their original trajectory is less clear. Using live cell imaging in zebrafish we show that within hours following nerve transection Schwann cells change morphology, engulf debris and migrate, yet remain closely associated with the original path. Concomitantly, growth cones sprout from the proximal stump, probe their environment, cross the transection gap, and eventually extend along denervated Schwann cells delineating the original path. In mutants lacking Schwann cells growth cones sprout and extend at rates

comparable to wild type, but fail to identify their original path, and instead extend along aberrant trajectories. To determine whether Schwann cells function primarily as a substrate we asked whether a Schwann cell-less axonal scaffold along the original path is sufficient to direct axonal growth. Even scaffolds bridging the injury gap failed to compensate for the absence of Schwann cells, providing compelling evidence that Schwann cells direct regenerating axons towards their original trajectory. To identify signals that guide regenerating axons in vivo, we examined mutants lacking the DCC guidance receptor. In DCC mutants a significant fraction of regenerating motor axons extended along aberrant trajectories, similar to the absence of Schwann cells. Thus, Schwann cells and DCC mediated guidance are critical early during regeneration, directing growth cones onto their original axonal trajectory.

8. Poor motoneuron development results in motor circuit defects in a vertebrate model of SMA. ***Christine Beattie***, *Hao Le, Duy Phan.* Neuroscience, Ohio State University, Columbus, OH.
We are interested in understanding how motoneurons develop in the infant/childhood motoneuron disease spinal muscular atrophy (SMA). Decreased levels of the survival motor neuron (SMN) protein is linked to this disease. To determine how motoneurons develop under conditions of low Smn, we generated maternal:zygotic *(mz)-smn* zebrafish mutants and analyzed motoneurons and other components of the motor circuit. We found that depletion of Smn resulted in motoneurons with fewer and shorter dendrites and thin motor axons with decreased arbors and fewer filopodia indicating that Smn is needed for proper motoneuron development. In addition, these defects resulted in decreased movement. Using a conditional transgene driving human SMN, we determined that we could rescue these defects only when we added SMN back soon after gastrulation. To address whether these defects were motoneuron cell autonomous, we developed a transgenic zebrafish line expressing human SMN in motoneurons using the *hb9/mnx1* promoter. By crossing the transgene into mutants, we found that we could completely rescue the motoneuron defects in *mz-smn* mutants indicating that Smn is needed cell-autonomously in motoneurons for their proper development. Our data also show that Smn is present in motor axons when they are activity growing suggesting a unique role for Smn during axon development. We next observed that the DRG sensory ganglia did not develop properly in *mz-smn* mutants. Their peripheral axons were shorter and often did not extend distally and the ganglia had fewer cells as development proceeded. We also found that Schwann cells were aberrant in *mz-smn* mutants. They failed to tightly associate with motor axons and had expanded Nodes of Ranvier. Strikingly, both the DRG and Schwann cell defects were rescued in *mz-smn* mutants by expressing SMN in motoneurons. Analysis of the DRG peripheral axon showed that it extends along the same region of the myotome as the motor nerve suggesting that poor motor axons impair the ability of the DRG peripheral axon to reach its target tissue. We conclude from these data that the deficit in motoneurons is a primary problem in SMA and results in secondary defects in other components of the motor circuit.

9. Wnt signaling mediates spinal cord regeneration by radial glia. *Lisa K. Briona,* ***Richard I. Dorsky***. Neurobiology and Anatomy, University of Utah, Salt Lake City, UT.
In mammals, spinal cord injury results in permanent sensory-motor loss due in part to a failure in the re-establishment of local neurogenesis. However, zebrafish show robust neuronal regeneration and functional recovery even after complete spinal cord transection. Postembryonic neurogenesis is dependent upon resident multipotent progenitors, which have been identified in multiple vertebrates. In the zebrafish spinal cord, the identity of these progenitors and the signals regulating their behavior remain uncharacterized. Using transgenes to both directly label cells and perform Cre-mediated lineage tracing, we have found that radial glial progenitors persist in the zebrafish spinal cord after embryogenesis. By employing a larval spinal cord transection assay, we have shown that these radial glia exhibit a proliferative and neurogenic response to injury, and contribute newly-born neurons to the regeneration blastema. Furthermore, our data show that the proliferation and differentiation of radial glia are highly correlated with axonal regrowth and functional recovery. The Wnt/b-catenin signaling pathway has been implicated in multiple regenerative processes, but the function of this pathway in CNS regeneration remains unclear. Our data show that Wnt activity is increased in radial glia within the regeneration blastema by one day after injury. Inhibition of Wnt signaling prevents injury-induced radial glial proliferation, blastema formation and neurogenesis, indicating that function of this pathway is essential for the regenerative response. Together, this work suggests that radial glia may be stem cells for neuronal regeneration following spinal cord injury in zebrafish, and that a fundamental part of the injury response includes Wnt pathway activation in radial glial progenitors.

10. Epidermal Cells Clear Sensory Axon Debris. ***Jeffrey P. Rasmussen***, *Georgeann Sack, Alvaro Sagasti.* Department of Molecular, Cell and Developmental Biology, UCLA, Los Angeles, CA.
Cellular debris generated by injury must be efficiently removed to maintain tissue homeostasis and prevent excess inflammation. We are using the larval zebrafish somatosensory system as a model to understand how axon debris is cleared following injury. Trigeminal and Rohon-Beard somatosensory neurons innervate the epidermis with elaborate peripheral axon arbors that detect touch stimuli. Injury to these arbors initiates a program of axon destruction called Wallerian degeneration (WD), which ultimately leads to the fragmentation of the damaged axon. In most examples of WD, macrophages, neutrophils and glia phagocytose axon fragments; however, when we genetically removed these cell types in zebrafish, the rate of clearance of degenerating somatosensory axon endings did not change. Electron microscopy showed that epidermal cells form pseudopodia that associate with axon debris during WD, leading us to hypothesize that epidermal cells phagocytose sensory axon debris. To visualize epidermal phagocytosis in living animals, we developed Gal4 transgenes specific for each layer of the larval epidermis and a UAS-driven transgenic reporter for phosphatidylinositol 3-phosphate [PI(3)P], a marker of phagosomes. Live imaging and automated tracking revealed that the epidermis phagocytoses at least 97% of axon fragments. Remarkably, most debris was phagocytosed within 8 minutes. Similar to phagocytosis in macrophages, axon fragments within the skin colocalized with markers of early and late endosomes and lysosomes, indicating that the epidermis degrades the axon debris. To test if the epidermis can phagocytose other types of cellular debris, we damaged axons within the posterior lateral line nerve (PLLn). PLLn axon debris is normally phagocytosed by leukocytes, but when we genetically mislocalized PLLn axons to the skin and induced WD, epidermal cells phagocytosed PLLn debris. Epidermal cells also ate debris following damage to neighboring epidermal cells. These results suggest

that the vertebrate epidermis has broad and previously unappreciated phagocytic abilities, which are likely important for neural repair and epidermal wound healing.

11. A zebrafish model for notochord-derived chordoma tumors as platform for drug screening and mechanistic studies of its tumor modifiers. ***Alexa Burger***[1,5,8], *Vasilyev Vasilyev*[2,4,7], *Ritu Tomar*[2], *Martin K. Selig*[4], *G. Petur Nielsen*[5], *Randall T. Peterson*[1,3,6], *Iain A. Drummond*[2], *Daniel A. Haber*[1,5], *Christian Mosimann*[8]. 1) MGH Cancer Center, Harvard Medical School, Charlestown, MA; 2) MGH Division of Nephrology, Harvard Medical School, Charlestown, MA; 3) MGH CVRC, Harvard Medical School, Charlestown, MA; 4) MGH Department of Pathology, Harvard Medical School, Charlestown, MA; 5) HHMI; 6) Broad Institute, Cambridge, MA; 7) Department of Biomedical Sciences, NYIT COM, Old Westbury, NY; 8) Institute of Molecular Life Sciences, University of Zurich, Switzerland.
Chordoma is a malignant tumor that is hypothesized to arise from remnants of embryonic notochord cells. The tumor is known to be highly chemo-resistant with few proven systemic therapies available for cases with unresectable disease or distant metastases. To date, genetic studies have not revealed recurrent mutations that may be targeted therapeutically, and pre-clinical screening for novel drugs has been limited by the absence of genetic model systems. We recently established a novel zebrafish model of chordoma driven by notochord-specific GFP-tagged HRASV12 expression. Our zebrafish model is histologically comparable to human chordoma and features a rapid onset that is well-suited for drug screening. We demonstrated a partial response to the mTORC1 inhibitor rapamycin, with a delay in the onset of the tumor phenotype and extension of survival. The successful generation of a zebrafish chordoma model using notochord-targeted expression of HRASV12 through the UAS/Gal4 system also establishes a general strategy for ectopically expressing other oncogenes implicated in chordoma, including the mesoderm transcription factor Brachyury. We are extending our current chordoma model with next-generation Tamoxifen-controlled CreERT2/loxP transgenes for spatio-temporal control of chordoma candidate genes using our growing collection of mesoderm-specific CreERT2 drivers. We have also started to induce genetic lesions implicated in chordoma formation using enhanced CRISPR/Cas9 protocols. Our findings will be instructive to establish diagnostic markers and therapeutic compounds towards curing human chordoma.

12. Yap Reprograms Glutamine Metabolism and Supports Growth During Liver Development and Tumorigenesis. ***Andrew Cox***[1,7], *Katie Hwang*[1,7], *Sebastian Beltz*[1], *Kimberley Evason*[2], *Keelin O'Connor*[1], *Evan Lien*[3], *Sagar Chhangawala*[4], *Yariv Houvras*[4], *Didier Stainier*[2,5], *Wolfram Goessling*[1,6]. 1) Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) University of California San Francisco, San Francisco, CA; 3) Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, MA; 4) Weill Cornell Medical College and New York Presbyterian Hospital, NY; 5) Max Planck Institute for Heart and Lung Research, Bad Nauheim. Germany; 6) Harvard Stem Cell Institute, Cambridge, MA; 7) Authors contributed equally.
The Hippo pathway has recently emerged as a key regulator of organ size and tumor formation. Perturbations of the Hippo pathway are often observed in various human cancers and can initiate tumorigenesis in mice. We developed transgenic zebrafish that express an activated form of the transcriptional coactivator Yap ($Yap1^{S87A}$), under the control of a hepatocyte-specific promoter (fabp10a). Embryonic liver size was greatly increased in Tg(fabp10a:$Yap1^{S87A}$) fish at 120 hours post fertilization. Hepatomegaly was maintained in adult Tg(fabp10a:$Yap1^{S87A}$) fish, which exhibited a 2-fold increase in liver:body weight ratio. The livers of Tg(fabp10a:$Yap1^{S87A}$) fish exhibited signs of dysplasia, but no frank cancers. Exposure of WT and Tg(fabp10a:$Yap1^{S87A}$) juveniles to the chemical carcinogen DMBA provoked liver tumor development, and Tg(fabp10a:$Yap1^{S87A}$) fish exhibited accelerated tumor formation. In order to identify genes that may contribute to the proliferative properties of Yap prior to tumor formation, we performed RNA-seq in WT and transgenic adult livers. Expression of genes involved in nitrogen metabolism were significantly altered. Particularly striking was a 10-fold increase in glutamine synthase, a key regulator of nitrogen metabolism. Metabolomic analysis of transgenic livers revealed that urea cycle intermediates were decreased while glutamine was increased. We conclude that Yap induces metabolic reprogramming in the liver, resulting in decreased NH_3 detoxification (Urea cycle) and increased NH_3 assimilation into glutamine. We hypothesize that the Yap-driven accumulation of glutamine may provide essential components for cell proliferation that may contribute to hepatic growth in liver development and tumorigenesis.

13. Disruptions to the growth and proliferation of zebrafish intestinal mutants are mediated by distinct transcriptomes. ***Joan Heath***[1], *Tanya de Jong-Curtain*[1], *Karen Doggett*[1], *Janine Coates*[1], *Julia Greisbach*[1], *Christopher Love*[1], *Oliver Sieber*[1], *Aliaksei Holik*[1], *Mirana Ramialison*[2]. 1) Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Victoria, Australia; 2) Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria 3008, Australia.
The overarching goal of our work is to identify genes and pathways that are indispensable for the growth of highly proliferative tissues that could potentially be targeted in novel therapeutic approaches to cancer, particularly colon cancer. To this end, we have been focusing on a collection of zebrafish intestinal mutants in which the growth of tissues that are rapidly proliferating in wild type zebrafish larvae at 72hpf is disrupted. The affected tissues include the intestinal epithelium, liver, exocrine tissue of the pancreas, craniofacial complex and discrete regions of the eye and brain. Meanwhile, most other tissues in the developing mutant larvae appear wild type. Positional cloning of the underlying mutated genes in this group of mutants has highlighted the essential requirement of components of large nuclear or cytoplasmic complexes that mediate key cellular processes, including transcription, mRNA and rRNA processing, nuclear pore formation and protein transport. However, rather than converging on a common stress response pathway, disruptions to these processes trigger quite distinct cellular responses, including apoptosis, cell cycle arrest and autophagy. In order to delineate the genetic events that produce these differential responses, we have carried out genome-wide transcriptome analysis at 72hpf using RNA-seq. Using locally developed state-of-the-art software for differential expression and gene set enrichment analysis (Subread, edgeR, ROAST), we have found that each mutant exhibits a unique transcriptome containing differentially regulated genes that may be key players in determining the nature of the stress response, and its dependence on p53 activation. The potential usefulness of a sub-set of these genes as targets for cancer therapy is currently being evaluated in tumour-prone mice.

Abstracts

14. A scalable system for studying metastasis using zebrafish. ***Richard M. White***[1], *Kajan Ratnakumar*[1], *Silja Heilmann*[2], *Erin Langdon*[3], *Emily Kansler*[1], *Elizabeth Perry*[1], *Joao Xavier*[2]. 1) Cancer Biology & Genetics, Memorial Sloan Kettering Cancer Center, New York, NY; 2) Computational Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 3) Dartmouth College, Hanover, NH.
Metastasis is the defining feature of advanced malignancy, yet its etiology and physiological consequences remain poorly defined. Melanoma, a tumor of transformed melanocytes, causes rapid death once metastatic, highlighting the need to further understand its mechanistic basis. We previously developed a zebrafish model of melanoma in which the human $BRAF^{V600E}$ gene is driven under the melanocyte specific mitfa promoter. When crossed with $p53^{-/-}$ animals, the resultant BRAF;p53 transgenics develop a 100% penetrant melanoma within 12 months. To define the metastatic capacity of this model, we derived ZMEL1, a GFP+ zebrafish melanoma cell line, and then transplanted that line into the casper recipient. Using a customized imaging algorithm, we developed a quantitative score of metastatic capacity after transplantation, taking into account the size of the primary tumor, and the number and distance of each of the subsequent metastases. This revealed that ~50% of recipient animals develop distant metastases, to organs such as the kidney marrow, muscle, liver and eye. The likelihood of metastasis, as expected, varies with the number of transplanted cells, and regression analysis revealed a Metastasis Initiating Cell (MIC) frequency of 1/250,000 cells in the ZMEL1 line. To avoid any need for immunosuppression of recipient animals, we can now transplant ~100 ZMEL1 cells into 2 day post fertilization embryos, and several weeks later nearly 100% of animals will have widely disseminated melanoma, making it highly amenable to screening approaches. To test this possibility, we have developed methodologies for nucleofecting expression vectors into the ZMEL1 line, allowing for direct testing of candidate metastasis factors such (i.e. SNAIL, NEDD9) in this assay. In the future, we plan to use this system for rapidly identifying both cell intrinsic and microenvironmental regulators of metastasis, which will directly contribute to our understanding of the human disease.

15. Atm and Phosphohistone H2ax are Required for Synchronization of Early Cell Cycles in Vertebrate Embryos. *Daniel Verduzco, Khalisa Syeda, Joshua Pierce,* ***James Amatruda****.* Departments of Pediatrics and Molecular Biology, Univ. of TX Southwestern Med Ctr, Dallas, TX.
The genome of all organisms faces threats from endogenous and exogenous sources of DNA damage. In response, organisms have evolved signal transduction and gene expression networks whose role is to detect, respond to and repair damage to the DNA. Deficiencies in these mechanisms are associated with degenerative diseases and cancer. While the mechanisms of the DNA damage response are relatively well-characterized, the roles of this network in early development are much less understood. In zebrafish, the first 12 cell cycles are rapid and synchronous, with the first cell division occurring about 30 minutes after birth. Each subsequent cycle completes in about 25 minutes. Desynchronization and lengthening of the cell cycle ensues during mid-blastula transition (MBT). Pre-MBT cell cycles are thought to lack a DNA damage-checkpoint, as they fail to arrest in the presence of DNA double-strand breaks. We previously showed that deficiency of the Ataxia-telangiectasia mutated (Atm) kinase, a key mediator of the DNA damage response, causes developmental defects and early lethality in zebrafish even in the absence of exogenous DNA damaging agents (Verduzco et al., Mol Canc Res 2012). To understand the roles of the DNA damage pathway in early cell cycles, we developed and validated an antibody specific for the phosphorylated form of Histone H2AX, a variant histone that is phosphorylated by Atm at Serine 139 in the presence of DNA damage. Unexpectedly, we find that H2AX S139 is cyclically phosphorylated during the synchronous pre-MBT cell cycles. Phosphorylation is strongest during interphase, absent in mid-mitosis, and reappears during the subsequent telophase. Expression of non-phosphorylatable or phospho-mimic H2AX variants led to marked desynchronization of early cell cycles. Similarly, chemical inhibition of Atm, the major H2AX kinase, led to loss of early cell-cycle synchronization. Our results suggest that cyclic H2AX phosphorylation serves to synchronize early cell-cycle progression, and reveal a novel requirement for DNA damage response proteins in early embryonic development.

16. *In Vitro* Construction of a Vertebrate Embryo. ***Bernard Thisse****, Peng-Fei Xu, Nathalie Houssin, Christine Thisse.* University of Virginia, Charlottesville, VA.
Our lab has previously shown that engineering opposing gradients of BMP and Nodal is sufficient to induce all molecular and cellular mechanisms required to organize *in vivo* uncommitted embryonic cells of the zebrafish blastula animal pole into a fully developed secondary embryonic axis. Here we show that animal pole explants of early blastula instructed by BMP and Nodal gradients, when placed in a culture medium can develop *in vitro* well-differentiated embryoids. After one hour, explants become spherical and differentiate an EVL. By late blastula, a central cavity, similar to a blastocoel, appears in the explant and a protrusion forms resulting from the induction by Nodal of a STAT3 dependent attraction center toward which surrounding cells converge. At gastrula stage, cells from the central part of this protrusion internalize, resulting in the formation of a blastopore with a blastopore lip, which is patterned along the D/V axis by the BMP signaling gradient. At this stage, explants display the organization plan of a prototypical vertebrate with clear A/P and D/V axes, a blastocoel, an archenteron and a blastopore located in a posterior-dorsal position. After one day in culture, explants differentiate into embryoids that display a bilateral symmetry showing for example a well-developed brain, two differentiated eyes with retina and lens. In addition, when BMP and Nodal signals are applied in a way that mimics the position of these two morphogenic gradients in wild-type blastula, the instructed explants differentiate into embryoids that display a beating heart and spontaneous myotomal contractions indicative of a functional central nervous system. Altogether, our study demonstrates that extensive embryo morphogenesis can be obtained *in vitro* using only two instructive signals, BMP and Nodal, acting zygotically. This reveals that any requirement for activity of maternal dorsal determinants and for signals emitted by the YSL can be bypassed by instructing naive cells of the blastula with experimentally engineered Nodal and BMP signaling centers. These results open the door for instructing embryoid bodies made of pluripotent cells to induce formation of embryonic tissues *in vitro*.

17. Dissecting the molecular mechanism of Neural Crest Inhibition by Kctd15. ***V. Zarelli****, I. Dawid.* National Institute of Health, Bethesda, MD, USA.
The neural crest (NC) is a multipotent migratory embryonic cell population that in vertebrates gives rise to multiple derivatives including the craniofacial skeleton, melanocytes and peripheral nervous system. Studies in zebrafish showed that the BTB-containing protein Kctd15 is a new antagonist of NC development, suggesting that it acts to delimit the NC and preplacodal domains at the neural plate boundary (Dutta and Dawid, Development 2010). In studying the mechanism of Kctd15 inhibition of NC formation we focused on the transcription factor AP-2 family (Zarelli and Dawid, PNAS 2013). AP-2 proteins are implicated in induction, differentiation, cell survival, and proliferation, and are essential regulators of NC formation. Kctd15 and AP-2a interact physically, and Kctd15 dramatically inhibits the activity of an AP-2 reporter in cultured cells and zebrafish embryos. Kctd15 binds to the activation domain (AD) of AP-2, and inhibits a fusion construct between the AD and the Gal4 DNA binding domain. Mutagenesis of proline 59 in the AP-2 AD led to an active protein that has lost Kctd15 binding and sensitivity. In contrast, mutating several neighboring prolines left activity and Kctd15 sensitivity intact. In zebrafish embryos, injection of Tfap2a WT fails but Tfap2a (P59A) mutant mRNA rescues the inhibition of NC development by Kctd15. Because of the relevance of the AP-2 activation domain in binding Kctd15, we hypothesized that Kctd15 may execute its functions by disturbing the recruitment of coactivators to the AP-2 complex. CBP/p300 and Cited2 have been described as AP-2 coactivators. In interaction studies we found that Kctd15 binds CBP/p300 but not Cited2. In reporter assays, excess of both CBP/p300 and Cited2 were not sufficient to rescue Kctd15 inhibition of AP-2 activity. Preliminary data implicate Ncoa2 (“nuclear receptor coactivator 2”) as a new coregulator that enhances AP-2 transcriptional activity. We observed a cooperative relationship between CBP/p300 and Ncoa2 in reporter assays favoring transcription stimulated by AP-2. Further studies are conducted to explore the cooperative role of ncoa2 with AP-2 during NC development and its participation in the restriction of the NC domain by Kctd15.

18. Dynamics and Shaping of the BMP Signaling Gradient by the BMP Antagonists During DV Axial Patterning. ***Joseph M Zinski***[1]*, Wei Dou*[2]*, David M Umulis*[2]*, Mary C Mullins*[1]. 1) Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA; 2) Department of Agricultural & Biological Engineering, Purdue University, West Lafayette, IN.
A gradient of Bone Morphogenetic Protein (BMP) signaling patterns the Dorsal-Ventral (DV) axis of the vertebrate embryo. However, its shape and dynamics during zebrafish DV patterning have not been described, nor quantified. Furthermore, how the extracellular BMP regulators shape the gradient in time and space is not known. To measure the BMP signaling gradient, we quantified the nuclear intensities of fluorescently stained Phosphorylated-Smad5 (P-Smad) in every cell of the embryo at 30-minute intervals from blastula through early gastrula stages. We used automated algorithms to identify the thousands of individual nuclei present at each embryonic time-point, and to measure their corresponding P-Smad intensities. In WT embryos, we show that the P-Smad gradient intensifies and steepens in the late blastula before stabilizing in the early gastrula. We compared the BMP signaling gradients in WT and BMP antagonist loss-of-function embryos of Chordin (Chd), Noggin (Nog), and Follistatin (Flst) to determine their spatiotemporal functions. Though Chd primarily inhibits BMP signaling, it has been shown to enhance BMP signaling by transporting BMP ligand in Drosophila DV patterning and crossvein formation. However, we find that Chd only inhibits BMP signaling laterally in DV patterning in the gastrula. This region specific inhibition allows Chd to steepen the BMP gradient. In contrast, loss of Nog and Flst have no effect on the gradient. However, the loss of all three antagonists causes a massive embryo-wide increase in BMP signal, far exceeding the lateral-only increase seen when Chd alone is absent. To elucidate mechanisms explaining these results, we generated a mathematical model of the system and screened 78,125 different networks, varying rates of production, diffusion, degradation, and binding. Our results suggest that: 1) in a WT system, Chd functions as the primary sink for BMP ligand. 2) When Chd is absent, Nog and Flst take over as the primary sink for the BMP ligand. 3) When all three are absent, no extracellular sink exists, and BMP signaling increases embryo-wide.

19. Wnt Promotes EMT and Mesodermal Cell Fate in the Zebrafish Tailbud via Znf703 and Cdh6. ***Brian Kinney****, Benjamin L. Martin.* Biochemistry & Cell Biology Dept., Stony Brook University, Stony Brook, NY.
The zebrafish tailbud contains a region populated by bipotential neural/mesodermal cells that serve as the primary source of posterior growth for embryos after the completion of gastrulation. These cells give rise to the posteriorly growing spinal cord, somites and blood vessels. Previous work has shown that Wnt signaling promotes mesodermal cell fate in the tailbud while repressing neural cell fate. However, the mechanism behind the mesodermal fate decision is unknown. Our results indicate that in the tailbud, Wnt signaling promotes mesoderm and represses neural cell fate in bipotental stem cells by inducing an epithelial to mesenchymal transition (EMT). Using heat shock inducible whole embryo assays and cell transplantation, we found the mechanism of Wnt induced EMT in the tailbud involves activation of the transcriptional repressor *znf703*, which disrupts cell adhesion by repressing the classical cadherin *cdh6*. Our evidence suggests that Znf703 represses *cdh6* indirectly through repression of the neural progenitor gene *sox2*. These results provide a mechanistic understanding of Wnt regulated mesoderm induction. Wnt signaling is required for mesoderm induction in all vertebrate embryos, and has also been shown to have diverse roles in stem cell development and cancer. This, along with the recent identification of *znf703* as a breast cancer oncogene, supports the use of the zebrafish tailbud as an excellent model to further elucidate the mechanisms of stem cell fate specification and cancer pathogenesis.

20. *cdc25a* times the cell cycle to facilitate mesodermal cell differentiation during posterior body formation in zebrafish. ***Cortney Bouldin***[1]*, Corey Snelson*[1]*, Gist Hank Farr*[1,2]*, David Kimelman*[1]. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA.
During the early stages of vertebrate body formation, an embryo grows from the head to the tail to generate the anterior-posterior (AP) axis. Throughout embryo elongation, the hind end of the embryo maintains a population of undifferentiated cells that provides the raw cellular material for posterior body formation and a population of differentiating cells that will contribute directly to the growing AP axis. As elongation is completed, the numbers of undifferentiated cells are winnowed to depletion. To prevent premature depletion of the

undifferentiated cells, both the numbers and the rate of release must be kept in balance until the AP axis is finished. At the onset of this study, we hypothesized that careful control of proliferation in the undifferentiated cells is critical for the completion of the vertebrate body. Through single cell fate-mapping experiments, we have found that proliferation in the undifferentiated cells can be divided into an early-rapid phase followed by a late-quiescent phase. The two phases of proliferation are matched by expression of the mitotic phosphatase, *cdc25a*. In undifferentiated cells, *cdc25a* is expressed during the early-rapid phase followed by depletion of *cdc25a* during the late-quiescent phase. Further by misexpressing *cdc25a* during the late-quiescent phase, we have found that if expression of *cdc25a* and proliferation are not restricted the ability of undifferentiated cells to proceed through mesodermal differentiation and contribute to the AP axis is blocked.

21. *Tmem18* is a regulator of adipose tissue development in zebrafish *in vivo*. ***Kathrin Landgraf***[1,2], *Roy Tauscher*[1], *Antje Berthold*[1], *Wieland Kiess*[1], *Antje Körner*[1,2]. 1) Center for Pediatric Research Leipzig (CPL), Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany; 2) Medical Center Adiposity Diseases (IFB), Leipzig, Germany.
Obesity affects nearly 500 million people worldwide and is associated with severe comorbidities, e. g. type 2 diabetes and cardiovascular complications. Genome-wide association studies identified SNPs associated with obesity, implicating many genes for a role in adipocyte biology but the physiological relevance of those genes has not been resolved. We have identified *TMEM18* as a regulator of human adipogenesis *in vitro*. The aim of this study was to investigate a potential role of *TMEM18* during adipose tissue development in zebrafish *in vivo*. We characterized the zebrafish ortholog of *TMEM18* and analyzed its expression from embryo to adult by quantitative *real-time* PCR and *in situ* hybridization. We assessed the effect of high-fat diet (HFD)-induced obesity on zebrafish *tmem18* expression. Finally, we analyzed adipocyte development after morpholino-mediated knockdown of *tmem18*. Similar to human, zebrafish *tmem18* was expressed in adult adipose tissue. Adipose tissue expression of *tmem18* was decreased in the state of HFD-induced obesity (63%±0.6%; $p<0.001$). *In situ* hybridization on early developmental stages revealed that *tmem18* was mainly expressed in the brain and the region surrounding the swim bladder where also expression of *pparg* was detected. *Tmem18* was upregulated at day 9 (3-fold compared to day 1, $p<0.001$). At this stage, first visceral adipocytes were detected near the swim bladder of the zebrafish larvae by Nile red staining. Injection of two independent *tmem18*-specific morpholinos into zebrafish oocytes resulted in a significant reduction in the number of adipocytes at 9 days post fertilization which was accompanied by a significant downregulation in *pparg* expression. Morpholino-mediated inhibition of *tmem18* expression did not affect zebrafish development in general. Moreover, there was no effect of *tmem18* knockdown on eating behaviour of zebrafish larvae suggesting that the inhibition of adipocyte formation is not mediated by a central effect. Our findings may indicate a potential role of *TMEM18* in adipogenesis *in vivo*.

22. p53-Independent Gene Regulations Are Critical for The Lysosomal Pathogenesis and Developmental Senescence in Spns1-Deficent Zebrafish. *Sasaki Sasaki*[1], *Shanshan Lian*[1], *Daniel Klionsky*[2], ***Shuji Kishi***[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) University of Michigan, Ann Arbor, MI.
Spinster homolog 1 (Spns1) in vertebrates is a lysosomal carbohydrate transporter that functions at a late stage of autophagy. Loss of *spns1* leads to the accumulation of enlarged autolysosomes that fail to degrade their contents, accompanied by increased lipofuscin accrual and senescence-associated b-galactosidase induction. However, little is known about the molecular genetic mechanism leading to the pathogenesis caused by such enlarged autolysosomes in vertebrates *in vivo*. To obtain robust hallmarks of senescence in zebrafish embryos, we examined the expression of other markers and/or mediators of senescence in *spns1*-defective animals. A gene expression profile in individual embryos demonstrated that the expression of *p21*, *plasminogen activator inhibitor-1* (*pai-1*), and *damage-regulated autophagy modulator 1* (*dram1*), which are known downstream targets of the p53 pathway, were upregulated in *spns1* morphants and mutants. *Senescence marker protein-30* (*smp-30*) was downregulated in *spns1*-deficient animals compared with the corresponding controls. While the induction of *p21* as well as *bax* was apparently regulated in a p53-dependent manner, both *pai-1* and *dram1* induction as well as the *smp-30* reduction in *spns1* mutants were not influenced by the p53 defect. Importantly, the suppression of *beclin 1* significantly counteracted the impact of *spns1* depletion by restoring expression of the *pai-1*, *smp-30*, and *dram1* genes. As described above, even in the absence of p53, the altered regulation of these critical gene markers was still detectable in *spns1*-deficient animals, indicating that p53-independent regulation may be responsible for the gene expression. In contrast, the induction of *p21*, *bax*, and *mdm2* genes in the *spns1*-defective condition was apparently p53 dependent and UV responsive, as confirmed by the level of their expression in p53 mutants. Taken together, the upregulation of *p21*, *pai-1* and *dram1* as well as the downregulation of *smp-30* in *spns1*-defective fish embryos may be consistent with the induction of autophagic abnormalities and senescence characteristics in aging organisms.

23. Knockout Zebrafish Model for the Understanding of Prolactin Action. *Yuqing Shu, Qiyong Lou, Jiangyan He,* ***Zhan Yin***. Institute of Hydrobiology, Chinese Academy of Sci, Wuhan, Hubei, China.
Prolactin (Prl) is an anterior pituitary hormone with broad ranges of functions. Its capabilities of lactogenesis, maternal behavior, growth and development, osmoregulation and epithelial ion transport have been reported in many vertebrates. In our present studies, zebrafish prl1 locus (ENSDARG00000037946) has been targeted via transcription activator-like effector nucleases (TALEN). Originally, a 210 amino acid peptide is encoded by the prl1 gene. Two mutant lines were generated by targeting the position between 3rd and 4th amino acids from N-terminus using TALEN procedure. The mutation sites were confirmed by DNA sequencing of the prl1 locus. The depletions of the Prl1 protein have also been confirmed by western blot analyses using specific antibody against carp Prl. All prl1-/- zebrafish progenies died during the 4-16 days post-fertilization stage in regular egg medium. However, the prl1-/- zebrafish embryos can be raised to adulthood with the salinity at 5000 mg/L for the fish culture water, which is equivalent to briny water with its salinity 90 times higher than the one of normal culture media. Our results have also demonstrated that no obvious defects, including growth and reproduction, have been observed in the prl1-/- zebrafish once they were kept in briny water. This clearly indicates the function of Prl in fresh water fish is mainly involved in the osmoregulation. The regulatory mechanisms of zebrafish Prl1, including ion transportation currently have been under investigation in

the laboratory. Our study here certainly provides valuable evidence for the understanding of the mechanisms of the Prl in phylogenetic and physiological perspectives.

24. Type 2 Diabetes in a Zebrafish Model of Insulin Resistance. ***Lisette Maddison***[1], *Ryan Kammeyer*[2], *Kaitlin Joest*[1], *Wenbiao Chen*[1]. 1) Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN; 2) Indiana University School of Medicine, Indianapolis, IN.
Type 2 diabetes (T2D) is a result of insufficient insulin production by the b-cells of the pancreatic islet. Although obesity is the primary risk factor for T2D, most obese individuals have normal glucose homeostasis despite being insulin resistant. Defects in b-cell compensatory mechanisms and b-cell protection likely contribute to the development of diabetes. With its genetic, chemical, and anatomical tractability, zebrafish is poised to help elucidate these mechanisms. To investigate the transition from insulin resistance to T2D, we generated a transgenic model with skeletal muscle-specific insulin resistance, zMIR. Under a regular feeding regimen, zMIR fish have significantly more b-cells than control siblings from 14 days onward, indicating compensation to insulin resistance. As a result, zMIR fish have normal glucose tolerance through 6 months of age. At 1 year of age zMIR fish have impaired glucose tolerance, indicating b-cell dysfunction, and reduced b-cell mass when compared to younger zMIR animals. When larvae were reared under an overnutrition paradigm, zMIR and control larvae initially have a similar expansion of the b-cell number. Following the 3rd day of overnutrition, the b-cell number declined substantially only in zMIR larvae and these larvae have an elevated level of free glucose. Three different classes of anti-diabetic drugs have a protective effect on the b-cells in the zMIR larvae. Furthermore, the b-cell loss occurs rapidly over a period of 4 hours, through a mechanism that does not involve b-cell apoptosis. Rather, there is a strong indication that the b-cell loss is through a phagocytic immune-cell mediated mechanism with macrophages having a dominant role. The dynamics of b-cell number in this zebrafish model, both in larvae and in adult animals, resembles pathogenesis of T2D in humans, setting forth a system to study mechanisms that are involved in the development of the disease.

25. Diabetes gene Hnf1b is directly required for hepatic gluconeogenesis response to insulin signaling. ***Joseph J. Lancman****, Keith P. Gates, P. Duc Dong.* Sanford Burnham Medical Research Institute, La Jolla, CA.
HNF1B is implicated in both early onset diabetes (MODY5) and Type 2 Diabetes. However the mechanism of HNF1B diabetes is not clear. We have previously reported a hypomorphic *hnf1ba* zebrafish mutant that exhibits a variable hypoplastic pancreas defect, the only current vertebrate model to phenocopy MODY5 pancreas phenotypes. These mutants also exhibit a specific and consistent reduction of b-cell numbers, which is a hallmark of diabetes. Although Hnf1b is known to be expressed in the hepatic ducts, our Hnf1ba immmunohistochemistry and BAC reporter studies reveal lower level expression in the hepatocytes, a major site of gluconeogenesis (GNG). The GNG pathway generates glucose to prevent hypoglycemia and is inhibited by insulin signaling. We find that *hnf1ba* mutant hepatocytes fail to down regulate the expression of a rate limiting enzyme of GNG, at the transcriptional level, in response to glucose/insulin treatment. Our mechanistic analyses suggest that Hnf1ba binds to an evolutionarily conserved site in the promoter of this gene to epigenetically repress transcription in response to insulin signaling. Consistently, adult *hnf1ba* mutants exhibit hyperglycemia, which we attribute to both reduced b-cell numbers and reduced hepatic insulin sensitivity. Our ongoing efforts are focused on identifying small molecules that can rescue these diabetic pathologies in our zebrafish mutant MODY5 model. .

26. Hecate/Grip2a acts to reorganize the cytoskeleton in the symmetry-breaking event of embryonic axis induction. *X. Ge*[1], *D. Grotjahn*[1], ***E. Welch***[1], *J. Lyman-Gingerich*[1], *C. Holguin*[1], *E. Dimitrova*[1], *EW. Abrams*[2], *T. Gupta*[2], *FL. Marlow*[2], *T. Yabe*[1], *A. Adler*[1], *MC. Mullins*[2], *F. Pelegri*[1]. 1) Genetics, University of Wisconsin-Madison, Madison, WI; 2) Perelman School of Medicine, University of Pennsylvania.
Axis induction requires the transport of dorsal determinants from the vegetal pole of the egg to the prospective organizer in the animal region. We previously reported that the maternal-effect gene *hecate*, which when mutated causes axis induction defects, encodes *glutamate receptor interacting protein 2a (grip2a)*, and that *grip2a* mRNA localizes to the vegetal pole of the embryo. *grip2a* mRNA localization is initiated during oogenesis in a process dependent on the Balbiani body. Upon fertilization the mRNA localization domain undergoes an off-center shift consistent with a recently proposed cortical rotation-like process. *hecate* mutants also exhibit defects in the off-center shift of *wnt8a* mRNA, a proposed dorsal determinant, and show aberrant distribution of the kinesin-associated cargo adaptor Syntabulin. We find that *hecate* mutants fail to exhibit the characteristic alignment and bundling of microtubules at the vegetal cortex thought to drive cortical rotation. This indicates that the site of action of *hecate* occurs at an upstream step in the axis induction pathway, and reveals a previously unrecognized role for *grip*-family genes in cytoskeletal reorganization. Although the short-range shift of vegetal factors is affected in *hecate* mutant embryos, mutants exhibit normal long-range, animally directed transport of injected beads along lateral regions of the yolk cortex. Furthermore, we show that such animally-directed movement along the lateral cortex is not restricted to a single arc corresponding to the prospective dorsal region. Together our results reveal a role for Grip2a in the reorganization of microtubules at the vegetal cortex to mediate a symmetry-breaking short-range shift corresponding to the teleost cortical rotation. The slight asymmetry achieved by this directed process is subsequently amplified by a general cortical animally-directed transport mechanism that is neither dependent on *hecate* function nor restricted to the prospective dorsal axis.

27. Cooperative regulation of convergence and extension by Planar Cell Polarity and notochord boundary signaling. ***Margot L.K. Williams***[1], *Atsushi Sawada*[1], *Chunyue Yin*[2], *Terin Budine*[1], *Lila Solnica-Krezel*[1]. 1) Developmental Biology, Washington University, Saint Louis, MO; 2) Division of Gastroenterology, Hepatology and Nutrition Cincinnati Children's Hospital Medical Center Cincinnati, OH.
All animal embryos, which begin as a disc or sphere, form an elongated body axis with a head on one end and a tail on the other. This fundamental process of axial elongation is accomplished by convergence and extension (C&E) of embryonic tissues, a process driven by polarized cell behaviors such as cell migration and mediolateral (ML) intercalation. Although molecular regulation of ML cell polarity in embryonic tissues is largely attributed to planar cell polarity (PCP) signaling, there is evidence that signals from the notochord-somite

boundary (NSB) are also involved. The nature of this NSB signal and its relationship to PCP signaling are not understood. The Solnica-Krezel lab has identified the chromatin factor Ugly duckling (Udu)/Gon4l as a novel regulator of C&E in zebrafish. Maternal zygotic (MZ) *udu* mutant embryos display an extremely short anterior-posterior axis, and interestingly, abnormal NSB and reduced ML polarity of boundary-adjacent cells. Complete loss of *udu/gon4l* function enhances axis elongation defects in PCP mutant embryos, and PCP-dependent cell polarity appears to be unaffected in MZ*udu* embryos, suggesting that *udu/gon4l* regulates ML cell polarity in parallel to the PCP pathway. We found that expression of *slit3*, which encodes a repulsive ECM ligand, is increased in MZ*udu* embryos. Expression of Slit3 in the axial mesoderm of WT embryos and of its Robo receptors in the adjacent paraxial mesoderm predict a role for Slit-Robo signaling in formation of the normal NSB. Furthermore, overexpression of *slit3* disrupts NSB formation and C&E in zebrafish embryos, and exacerbates axis elongation defects in PCP mutants. We propose that Slit-Robo signaling at the NSB mediates a novel polarity cue that acts in parallel to PCP to regulate ML polarity and polarized cell intercalation that drive C&E.

28. Single lumen formation in the zebrafish gut is mediated by *smoothened*-dependent tissue remodeling. ***Ashley Alvers***[1], *Sean Ryan*[1], *Paul Scherz*[2], *Jan Huisken*[3], *Michel Bagnat*[1]. 1) Duke University Medical Center, Durham, NC; 2) University of California San Francisco, San Francisco, CA; 3) Max Planck Institute of Molecular Cell Biology and Biology and Genetics.
The formation of a single lumen during tubulogenesis is crucial for the development and function of many organs. Although 3D cell culture models have identified molecular mechanisms controlling lumen formation *in vitro*, their function during vertebrate organogenesis is poorly understood. Therefore, we used the zebrafish gut as a model to investigate single lumen formation during tubulogenesis. Previous work has shown that multiple small lumens enlarge through fluid accumulation and coalesce into a single lumen. However, since lumen formation occurs in the absence of apoptosis, other cellular processes are necessary to facilitate single lumen formation. Using light sheet microscopy and genetic approaches, we show that during gut development multiple lumens open and enlarge to generate a distinct intermediate, which consists of two adjacent un-fused lumens separated by basolateral contacts. We observed that these lumens arise independently from each other along the length of the gut and do not share a continuous apical surface. Resolution of this intermediate into a single, continuous lumen requires the remodeling of contacts between adjacent lumens and subsequent lumen fusion. We show that lumen resolution, but not lumen opening, is impaired in *smoothened (smo)* mutants, indicating that fluid-driven lumen enlargement and resolution are two distinct processes. Furthermore, we show that, *smo* mutants exhibit perturbations in the Rab11 trafficking pathway and demonstrate that Rab11-mediated trafficking is necessary for single lumen formation. Thus, lumen resolution is a distinct genetically-controlled process critical for single, continuous lumen formation in the zebrafish gut.

29. Rabconnectin-3a Regulates Vesicle Endocytosis and Canonical Wnt Signaling in Zebrafish Neural Crest Migration. ***Adam Tuttle***, *Trevor Hoffman, Thomas Schilling*. University of California, Irvine, Irvine, CA.
The neural crest (NC) is a population of cells in vertebrates characterized by an epithelial-to-mesenchymal transition (EMT) followed by multiple waves of migration to many parts of the developing embryo and gives rise to several distinct cell lineages. The initiation of NC cell EMT, migration, and path-finding requires a variety of signals, including canonical and non-canonical Wnts, and dynamic regulation of the expression and subcellular localization of cell-cell adhesion molecules, such as Cadherins. Both of these processes can be regulated by controlled endocytosis, lysosomal degradation, and recycling of ligand-receptor complexes and cell-cell adhesion molecules from the plasma membrane of migrating cells. We discovered a gene, rabconnectin-3a (rbc3a), with novel early NC expression in zebrafish, which when mutated disrupts migration of a subset of NC cells. Our data suggest that rbc3a is required for proper endosomal maturation independent of acidification in NC cells. Maturation and fusion of endosomes is known to regulate signaling pathways, such as Wnt, by aiding in the formation of multivesicular bodies (signalosomes) or contributing to lysosomal degradation of receptors and/or ligands. Loss of rbc3a function downregulates direct canonical Wnt targets necessary for NC EMT and migration during a critical time period in NC development. Furthermore, rbc3a-deficient NC cells that fail to migrate differentiate into pigment progenitors and display aberrantly high levels of Wnt receptor at the membrane with corresponding high levels of canonical Wnt signaling at later developmental stages. We propose a novel developmental role for Rbc3a in EMT of the NC, in which it acts, at least in part, through its regulation of Wnt signaling in both pre- and post-migratory NC cells and its requirement in early endocytosis.

30. Bmp15 is an oocyte-produced signal required for maintenance of the adult female sexual phenotype in zebrafish. ***D.B. Dranow***, *A.M. Bird, M.T. Adams, B.W. Draper*. Dept. of Molecular and Cellular Biology, University of California Davis, Davis, CA.
Previous work has established that meiotic oocytes are necessary for female development between about 25-35 days post-fertilization (dpf). We have since shown that not only are oocytes required during this early sex determination time point but are also required in adults to maintain the female sex. Our hypothesis is that the ability to remain female is dependent upon the amount of estrogen produced in the gonad and that signals produced by the oocyte act to increase or stabilize estrogen production by the somatic gonad. Estrogen is produced by the aromatase Cyp19a1, using androgens as a substrate. In mammals, Cyp19a1 is produced by granulosa cells that surround the oocyte. In zebrafish, the aromatase orthologue Cyp19a1a is expressed in the ovary, but the specific cell type has not been determined. Using BAC recombineering, we have produced a *Tg(cyp19a1a:EGFP)* reporter line and have found that *cyp19a1a* is not expressed in granulosa cells that surround stage I oocytes, but instead is expressed in the outer theca cell layer. By contrast, in stage II and later oocytes, it is expressed in both the theca and granulosa cell layers. The oocyte-produced factors Bmp15 and Gdf9 (growth differentiation factor 9) are two closely-related TGF-b ligands that in mammals are required for the proper development and function of granulosa cells. These factors are therefore candidates for regulating estrogen production by the somatic gonad. To test this hypothesis, we used TALENs to introduce frame-shift mutations in the *bmp15* and *gdf9* loci. Surprisingly we found that *gdf9* mutants are fertile and phenotypically wild-type. By contrast, however, *bmp15* mutant females can produce stage IB and early stage II oocytes during early larval development, but by 50 dpf, these oocytes begin to disappear as the gonad switches to a spermatogenic program. As a consequence, all *bmp15* mutant adults are phenotypic and functional males. We therefore conclude that Bmp15 is an oocyte-produced signal that is required for normal oocyte development and

therefore maintenance of the female sexual phenotype in adult zebrafish. We are currently determining how loss of Bmp15 signaling affects estrogen production by the somatic gonad.

31. Chemokine guided angiogenesis directs coronary vasculature formation in zebrafish. ***Michael RM Harrison***[1], *Jeroen Bussmann*[3], *Arthela Osorio*[1], *Arndt Siekmann*[3], *Ching-ling Lien*[1,2]. 1) Children's Hospital Los Angeles, Los Angeles, CA; 2) Keck School of Medicine, University of Southern California; 3) Max-Planck Institute for Molecular Biomedicine, Muenster, Germany.
Interruption of coronary blood supply severely impairs heart function with often-fatal consequences for heart disease patients. However the formation and maturation of these coronary vessels is not fully understood. Here we report the first detailed analysis of coronary vessel formation in zebrafish. We observe that coronary vessels form in zebrafish by angiogenic sprouting of endothelial cells that express the CXC-motif chemokine receptor Cxcr4a. These endothelial cells migrate to vascularize the ventricle under the guidance of the myocardium-expressed ligand Cxcl12b. cxcr4a mutant zebrafish fail to form a vascular network, whereas ectopic expression of Cxcl12b ligand induces coronary vessel formation. Importantly, cxcr4a mutant zebrafish fail to undergo heart regeneration following injury. Our results suggest that Cxcl12/Cxcr4 chemokine signaling has an essential role in coronary vessel formation by directing migration of endothelial cells. Poorly developed vasculature in cxcr4a mutants likely underlies decreased regenerative potential in adults.

32. Visnagin Protects Against Doxorubicin-Induced Cardiomyopathy Through Inhibition of Mitochondrial Malate Dehydrogenase. ***A. Asnani***[1], *Y. Liu*[1], *L. Zou*[2], *V. Bentley*[3], *M. Yu*[4], *Y. Wang*[1], *G. Dellaire*[3], *K.S. Sarkar*[1], *M. Dai*[1], *H.H. Chen*[1,5], *D.E. Sosnovik*[1,5], *J.T. Shin*[1], *D.A. Haber*[4], *J.N. Berman*[3], *W. Chao*[2], *R.T. Peterson*[1]. 1) Cardiovascular Research Center and Harvard Medical School (HMS), Massachusetts General Hospital (MGH), Charlestown, MA; 2) Department of Anesthesia, Critical Care and Pain Medicine, MGH and HMS; 3) Department of Pathology and Biochemistry & Molecular Biology, Dalhousie University, Halifax, Nova Scotia; 4) MGH Cancer Center and HMS; 5) Martinos Center for Biomedical Imaging, MGH and HMS.
Anthracyclines such as doxorubicin are used to treat a number of common malignancies. Doxorubicin exhibits potent tumoricidal activity but can also cause dose-dependent cardiotoxicity, leading to congestive heart failure. The molecular pathways responsible for doxorubicin cardiotoxicity have not been clearly defined, and most cardioprotective therapies to date have failed to demonstrate the clinical benefit necessary for routine incorporation into chemotherapy regimens. To tackle this problem, we established a zebrafish model of doxorubicin cardiotoxicity. Administration of doxorubicin to zebrafish larvae recapitulates human heart failure, resulting in decreased myocardial contractility and increased cardiomyocyte apoptosis. From a chemical screen of 3000 compounds tested in this model, our laboratory identified the small molecule visnagin (VIS) as a potential antidote to doxorubicin cardiotoxicity. Importantly, VIS preserves cardiac function in mice treated with doxorubicin but does not affect doxorubicin's tumoricidal activity in mouse and zebrafish xenograft models. Using an affinity chromatography approach, we discovered that VIS binds to mitochondrial malate dehydrogenase (MDH2), one of the key enzymes in the tricarboxylic acid (TCA) cycle. Treatment with the MDH2 inhibitors mebendazole, thyroxine, and iodine also prevents the development of doxorubicin cardiotoxicity in zebrafish, suggesting that MDH2 inhibition is responsible for visnagin's cardioprotective effects. In addition, treatment with L-malic acid itself protects against doxorubicin cardiomyopathy. This study identifies visnagin as a potent cardioprotective compound and implicates MDH2 as a novel drug target in doxorubicin-induced cardiomyopathy.

33. Asymmetric cell division precedes lymphatic endothelial precursor cell emergence from the cardinal vein in zebrafish. ***Kaska Koltowska***[1], *Anne Karine Lagendijk*[1,2], *Elke Ober*[3,4], *Alpha Yap*[2], *Benjamin M Hogan*[1]. 1) Divisions of Genomics of Development and Disease, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia; 2) Divisions of Molecular Cell Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia; 3) Division of Developmental Biology, MRC National Institute for Medical Research, London, UK; 4) The Danish Stem Cell Centre,University of Copenhagen, Copenhagen, Denmark.
Specification of progenitor cells is a key step in tissue differentiation and organogenesis. Transcription factors play an essential role in cell fate specification and restriction of tissue identity. During lymphatic vascular development in mammals, the transcription factor Prox1 marks specified lymphatic precursors in the cardinal vein (CV). These progenitors migrate out of the vein to form a lymphatic progenitor pool in response to Vegfc signalling. Interestingly, it is well established that asymmetric distribution of Drosophila prospero (the Prox1 orthologue) during cell division can establish cell fate in one daughter cell while the other remains in an unspecified state. In zebrafish, the onset of lymphatic specification and the underling molecular identity remains unclear. We used a zebrafish transgenic reporter line of prox1(1a) transcription (using KalT4, 4xUAS:tagRFP) to specifically label lymphatic endothelial cells (LECs) in zebrafish. By crossing the prox1 reporter onto 10xUAS:Venus, we observe strong expression in LECs at earlier stages than previously reported and we find that lymphatic specification occurs at the level of CV from 32hpf. This observation was supported by antibody staining against Prox1 protein. Time-lapse analysis of prox1:KalT4 1xXUAS:Venus during LEC specification revealed that precursors undergo cell division in the wall of the vein before emergence of LECs. One daughter cells remains in the vein and switches off Prox1 expression, while other cell migrates out of the vein to form a LEC. We show that asymmetric distribution of Prox1-positive precursors can drive LEC specification, answering one of the major open questions in the field.

34. The role of Nkx3.2 in vascular stabilization and neural crest migration. ***Thomas R Whitesell***, *Peter Spice, Jae-Ryeon Ryu, Sarah J Childs.* University of Calgary, Calgary, Alberta, Canada.
Vascular mural cells, consisting of either smooth muscle cells or pericytes, arise from a migratory neural crest origin in the head of all studied vertebrate models, though the process has not been well characterized in zebrafish. We are interested in studying how neural crest cells of zebrafish embryos are recruited to and stabilize nascent blood vessels. Our preliminary data indicate the lineage of mural cells in the ventral zebrafish head may not be solely neural crest derived, as some neural crest markers (Sox10, Fli1a) do not co-localize with the vascular mural cell marker (aSMA/Acta2). However, defects in neural crest specification or mural cell maturation are associated with hemorrhage, suggesting that brain mural cells may be neural crest derived. We have identified a transcription factor, Nkx3.2 / Bapx1,

which mediates vascular stabilization in zebrafish and regulates neural crest migration. Knockdown or mutation of Nkx3.2 results in hemorrhage, reduced mural cell coverage on blood vessels, and abnormal migration of neural crest cells. Nkx3.2 negatively regulates Sox10, and positively regulates Pdgfra, both of which are key players in neural crest migration and development. These results implicate Nkx3.2 as a mediator of neural crest development, culminating in mural cell recruitment and function. In addition, our data suggests there may be additional origins of vascular mural cells in the ventral head of fish, possibly from mesoderm. While we cannot rule out contribution to mural cell lineage from sources other than the neural crest, here we present evidence that regulation of key genes involved in migratory neural crest by Nkx3.2 is required for vascular stability through mural cell recruitment and maturation.

35. Role of FGF signaling in maintenance of cardiac chamber identity. ***Arjana Pradhan***[1], *Xin-Xin Zeng*[1], *Sara Marques*[2], *Neil Chi*[3], *Deborah Yelon*[1]. 1) Cell and Developmental Biology, UC San Diego, La Jolla, CA; 2) Developmental Genetics program and Department of Cell Biology, Kimmel Center for Biology and Medicine, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY; 3) Department of Medicine, Division of Cardiology, UC San Diego, La Jolla, CA.
The heart is composed of two types of cardiac chambers - atria and ventricles - with unique morphological, electrophysiological, and contractile properties. Therefore, proper chamber-specific differentiation and maintenance of the unique features of atrial and ventricular cardiomyocytes are essential for the formation of a functional heart. We recently reported an essential role for the transcription factors Nkx2.5 and Nkx2.7 in maintenance of ventricular chamber identity. However, little is known about the pathway in which Nkx factors operate to control retention of chamber-specific characteristics. Here, we show that the FGF signaling pathway, which facilitates ventricular specification, is also required after the initial differentiation of ventricular cardiomyocytes in order to preserve their ventricular identity. We find that both pharmacological and genetic inhibition of FGF signaling can generate ectopic atrial cardiomyocytes within the already differentiated ventricle. Analysis using photoconvertible transgenes suggests that these ectopic cells are produced through transdifferentiation of ventricular cardiomyocytes derived from the first heart field (FHF). Additionally, late-differentiating cardiomyocytes, derived from the second heart field (SHF), inappropriately adopt an atrial identity when FGF signaling is impaired. Furthermore, we find that nkx2.5 expression is reduced in embryos with compromised FGF signaling, suggesting that nkx2.5 acts downstream of FGF to promote ventricular maintenance. Together, our data suggest a model in which continuous FGF signaling is required to preserve ventricular identity in cardiomyocytes derived from the FHF and to suppress atrial identity in late-differentiating cells from the SHF.

36. Visualization, interaction, and integrative analysis of *in toto* images using GoFigure2. ***Kishore Mosaliganti***, *Arnaud Gelas, Lydie Souhait, Nicolas Rannou, Nikolaus Obholzer, Sean Megason.* Systems Biology, Harvard Medical School, Boston, MA 02115, USA.
In the last decade, systems approaches to the study of biology have significantly benefited from the application of *in toto* imaging technologies to the zebrafish model system. For example, in developmental science, with the aid of this approach, one can comprehensively digitize biological processes at sub-micron resolutions and across thousands of cells in live and intact embryos. *In toto* imaging produces information-rich datasets that are large and multi dimensional (xyz+t+l), capturing dynamic and mechanistic information across a range of spatial scales. To extract information from such datasets, we developed GoFigure2, a collaborative open-source platform for the visualization, interaction, and analysis of *in toto* imagery. Along with a large 5D image data model, GoFigure2 supports a trace data model consisting of 2D/3D surfaces, 4D cell trajectories, and lineage trees. Such a robust integration of image and trace data synergistically facilitates the development of novel interaction and visualization tools. Trace data can be obtained in the GoFigure2 framework using manual, semi-automated, or fully automated image analysis algorithms for cell segmentation, tracking, and lineaging. This enables the user to systematically quantify biological parameters across multiple scales (sub-cellular to tissues) and study it in the context of a single cell. As an example case study, we demonstrate the utility of GoFigure2 in understanding the complex morphogenetic steps involved in early ear development.

37. In situ hybridization using HCR: mRNA and miRNA targets, straightforward multiplexing, high signal-to-background, sub-cellular resolution, relative quantitation. ***Harry Choi***[1], *Vikas Trivedi*[1], *Aneesh Acharya*[1], *Victor Beck*[1], *Scott Fraser*[2], *Niles Pierce*[1,3]. 1) Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Translational Imaging Center, University of Southern California, Los Angeles, CA; 3) Division of Engineering & Applied Science, California Institute of Technology, Pasadena, CA.
Conventional in situ hybridization methods based on catalytic deposition of fluorescent dyes or colored precipitates yield high-contrast staining patterns within whole-mount zebrafish embryos, but require cumbersome serial multiplexing protocols and generate non-quantitative, low-resolution signal. These longstanding difficulties are overcome using in situ amplification based on the mechanism of hybridization chain reaction (HCR; Dirks & Pierce, *PNAS*, 2004). Here, we present the next-generation DNA HCR technology, dramatically increasing signal gain, reducing reagent cost, and improving reagent durability compared to the published RNA HCR approach (Choi *et al*., *Nature Biotechnol*, 2010). Using DNA HCR, probes complementary to mRNA or miRNA targets trigger chain reactions in which metastable fluorophore-labeled DNA hairpins self-assemble into tethered fluorescent amplification polymers. Within whole-mount zebrafish embryos, DNA HCR enables straightforward multiplexing (simultaneous signal amplification for multiple target RNAs), high signal-to-background (non-overlapping pixel intensity distributions for signal and background), high resolution (sub-cellular localization and colocalization of signal), and accurate relative quantitation (pixel intensities scale linearly with target abundance). HCR in situs provide the zebrafish research community with powerful new capabilities for the study of RNA expression in a morphological context. To assist researchers in adopting the technology, kits and technical support are available via the non-profit academic resource at www.molecularinstruments.org.

38. PyFRAP: An open-source software package for FRAP analysis. ***Alexander Bläßle***[1], *Patrick Müller*[1], *Ben M Jordan*[2]. 1) Friedrich Miescher Labrotory, Tübingen, Germany; 2) Department of organismic and evolutionary biology, Harvard University, Cambridge, MA, USA.
The transport of macromolecules is crucial for development and homeostasis and must be tightly controlled to prevent developmental defects and disease states. Macromolecules such as proteins must often move through complex environments from their site of production to their target site. What are the biophysical properties of such molecules, and how is their transport affected by the properties of the environment through which they move? One technique to address these questions is FRAP (Fluorescence Recovery After Photobleaching), which measures the mobility-driven recovery of fluorescent molecules in a photobleached area. Although FRAP assays have been used for 40 years, there are several shortcomings of current data analysis methods. Most analysis algorithms make simplified assumptions about the sample geometry, bleaching conditions, and the underlying kinetics of the system that do not fully reflect the complexities of the experimental sample. To address these shortcomings, we developed the Python software package "PyFRAP". PyFRAP numerically solves the equations that govern macromolecule transport on an exact three-dimensional model of the sample using finite element simulations. To account for bleaching inhomogeneities, PyFRAP matches the FRAP image data with the initial condition on the simulation. Simulated solutions are then fitted to the experimental data using a variety of optimization algorithms. We present two studies to illustrate the power of PyFRAP: In the first study, we use an in vitro FRAP assay for various sizes of fluorescent molecules in different environments. Using this in vitro assay, we show that PyFRAP produces reliable estimates of diffusivities comparable to other measurement techniques such as Fluorescence Correlation Spectroscopy (FCS). Secondly, we present a set of in vivo experiments in zebrafish embryos to measure the diffusivities of extracellular fluorescent molecules. PyFRAP comes with an intuitive graphical user interface that can be applied to a variety of systems to obtain quantitative insight into the biophysics of molecule movement.

39. Endoderm convergence controls subduction of myocardial precusors during heart-tube formation. ***Fang Lin***, *Ding Ye*. Anatomy and Cell Biology, University of Iowa, Iowa City, IA.
Vertebrate heart formation requires that bilateral sets of myocardial precursors migrate to the midline, where they form the primitive heart tube. We previously showed that myocardial migration is promoted by convergent movement of the endoderm, which depends on a S1pr2/Ga13-dependent signaling pathway. However, the underlying mechanisms remained unclear. Here, we report our discovery (based on a combination of in vivo and en site imaging approaches) of strikingly complex associations between the endoderm and myocardial precursors during their migration to the midline. During segmentation, the endoderm rapidly converged toward the midline through the 13-somite stage (13s); this migration then slowed to a minium. In contrast, the myocardial cells underwent three distinct modes of migration: 1) before 13s, they were located dorsal and next to the adjacent endoderm, engaging in passive migration towards the midline; 2) at 13s, they migrated to the ventral side of the endoderm (underwent subduction); and 3) after 13s, they engaged in active migration toward the midline beneath the endoderm. Although both passive migration and subduction depended directly on endoderm convergence, active migration did not; rather, the impairment of active myocardial migration in the context of defective S1pr2/Ga13 signaling resulted from hyper-epithelialization of these cells due to disruption of the earlier steps of myocardial migration. Collectively, our data demonstrate that the interplay between the endoderm and myocardial precursors during heart-tube formation is extensive, and highlight the importance of endoderm movement for proper heart development. Defects in the mechanisms reported here could underlie some congenital heart diseases.

40. Calcium-dependent Naked-Dishevelled Interaction Modulates Wnt Signalling Outputs. ***AN Marsden***[1,2], *SW Derry*[1], *TA Westfall*[1], *DC Slusarski*[1]. 1) Department of Biology, University of Iowa, Iowa City, IA 52242; 2) Interdisciplinary Graduate Program in Genetics.
The Wnt signaling network plays critical roles in development and is implicated in human disease. Wnts comprise a complex signaling network that, upon ligand binding, activates the phosphoprotein Dishevelled (Dvl), leading to distinct outputs including polarized cell movement (known as planar cell polarity, Wnt/PCP) and stabilization of the transcription factor b-catenin (Wnt/b-catenin). The mechanisms that determine a specific output is not completely understood, especially since they share receptors and cellular effectors. My project focuses on two such shared components that also bind each other, Dvl and Naked (Nkd). Previously we demonstrated that Nkd is required for zebrafish dorsal forerunner cell (DFC) migration, Kupffer's vesicle formation and proper organ laterality. Moreover, we identified calcium fluxes in the DFCs and determined that the EF-hand motif in Nkd weakly binds calcium. Using a combination of biochemical and functional assays, we show calcium-induced conformational changes in the Nkd-Dvl complex and identify a requirement for the Nkd EF-hand in cell polarity but not in b-catenin transcriptional outputs. We predict that Nkd and Dvl form a cooperative calcium binding pocket, which allows for conformational changes or subcellular localization to direct Wnt/PCP output. We identified a region in Dvl that may coordinate ion binding. We have mutated this novel Dvl calcium binding site, and performed biochemical, genetic, and functional studies. To determine the impact upon Wnt signaling output, I utilize gene knockdown and rescue in the zebrafish DFCs, in a tissue that hosts converging Wnt signals. I also determined the subcellular localization of Nkd and Dvl components within the cells known to have calcium fluxes and cells that are quiescent. Our data suggests that calcium-induced secondary structure changes in the Nkd-Dvl complex serve to interpret the physiology of a cell receiving multiple cues and provides mechanistic insight into Wnt signal integration in vivo.

41. Targeted knockout of a habenula gene increases anxiety and social cohesion. ***Cheol-Hee Kim***, *Jung-Hwa Choi*. Department of Biology, Chungnam National University, Daejeon, South Korea.
Emotional responses such as fear and anxiety are essential for decision-making and survival. The habenula (Hb) is a structure in the epithalamus highly conserved during vertebrate evolution, and mediates behavioral responses to stress, anxiety, and fear. Dysfunction of Hb is associated with depression, post-traumatic stress disorder, and schizophrenia in humans. Further, the ablation of Hb has been shown to profoundly alter fear and anxiety responses in fish and mice. However, the molecular mechanisms underlying the emotional responses

and functional regulation of Hb remain largely unknown. Here we report the critical function of a novel chemokine-like protein, Samdori-2 (Sam2), in the processes. We found that Sam2 is specifically expressed by neurons in the dorsal Hb of zebrafish. Targeted knockout (KO) of the sam2 gene by zinc finger nucleases in zebrafish did not affect viability, fertility, or general morphology. Furthermore, sam2 KO fish showed normal development of neural circuits including Hb to interpeduncular nucleus (IPN) pathways. However, sam2 KO fish exhibited strikingly elevated anxiety-like behaviors in the novel tank and scototaxis tests and showed increased social cohesion. In neurons, Sam2 showed neuromodulatory ability of strongly attenuating inhibitory synaptic functions. These results establish a crucial role of sam2 in regulating Hb-mediated anxiety and social cohesion. Furthermore, our studies uncover a new neuromodulatory mechanism mediated by novel chemokine-like proteins to control neuronal circuit function and behavior. In addition, I will introduce two more TALEN-based KO zebrafish showing infantile spasm and aggression behavior, respectively.

42. Habenulo-Raphe Circuit Transmits a Danger Avoidance Signal. ***R. Amo***[1,2], *F. Fredes*[1], *M. Kinoshita*[1], *R. Aoki*[1,3], *H. Aizawa*[1], *M. Agetsuma*[1], *T. Aoki*[1], *T. Shiraki*[1], *H. Kakinuma*[1], *M. Matsuda*[4], *M. Yamazaki*[1], *M. Takahoko*[1], *S. Higashijima*[5], *N. Miyasaka*[6], *T. Koide*[6], *Y. Yabuki*[6], *Y. Yoshihara*[6], *H. Okamoto*[1,2]. 1) Lab. for Developmental Gene Regulation, RIKEN Brain Science Institute, Saitama, Japan; 2) Dept. of Life Science and Medical Bioscience, Waseda Univ., Tokyo, Japan; 3) Dept. of Life Sciences, Graduate School of Arts and Sciences, Univ. of Tokyo, Tokyo, Japan; 4) Center for Bioscience Research and Education, Utsunomiya Univ., Tochigi, Japan; 5) National Inst. of Natural Sciences, Okazaki Inst. for Integrative Bioscience, NIPS, Aichi, Japan; 6) Lab. for Neurobiology of Synapse, RIKEN Brain Science Inst., Saitama, Japan.
Anticipation of danger at first elicits panic in animals but later it helps them to avoid the real threat adaptively (*e.g.* active avoidance). The neural circuit enabling such proactive use of danger expectation is unknown. A candidate site responsible for active avoidance is the lateral habenula (LHb). In mammals, LHb neurons are suggested to have a role in transmitting anti-reward and aversive information. The Hb is conserved in vertebrates, and the zebrafish homolog of the mammalian LHb, the ventral habenula (vHb) has a simple structure including an exclusive direct projection to the serotonergic median raphe (MR). Using adult zebrafish as a model, we found that tetanus toxin mediated genetic inhibition of synaptic transmission from the vHb to the MR impaired active avoidance learning, while Pavlovian fear conditioning remained intact. *in vivo* electrophysiolosy (loose-patch recording) from genetically labeled vHb neurons during pavlovian fear conditioning showed that the vHb neuronal activity generated an expectation signal for a dangerous context. Accordingly, artificially triggering an expectation signal by optogenetic stimulation of vHb neurons in free-moving fish evoked place avoidance. We also found that optogenetic stimulation of the vHb axons in acute slice activate genetically labeled serotonergic neurons in the raphe. Thus, the vHb-MR circuit is essential for presenting danger expectation and programming active avoidance. These results reveal how perceived risk in the environment is communicated to the serotonin system to avoid potential hazard.

43. Forward genetic screen reveals a role for the *pregnancy associated plasma protein-a* gene in habituation learning. ***Marc Wolman***[1,3], *Roshan Jain*[1], *Kurt Marsden*[1], *Hannah Bell*[1], *Katherina Hayer*[2], *John Hogenesch*[2], *Michael Granato*[1]. 1) Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine; 2) Pharmacology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; 3) Zoology, University of Wisconsin, Madison, WI.
The nervous system continuously integrates sensory information with previous experiences to select an appropriate behavioral response. A fundamental form of this integration process is habituation; a simple form of non-associative learning that is conserved from *Aplysia* and *C. elegans* to mammals. Habituation is defined as response suppression to repeated, inconsequential stimuli and serves as a mechanism to filter irrelevant information. Unbiased, large-scale systematic approaches in invertebrate organisms have yielded great insight into the genetic regulation of behavior. However, for many reasons, this approach has been difficult to recapitulate in mammalian vertebrate organisms, even for simple forms of cognition like habituation. Larval zebrafish show a remarkable capacity for behavioral plasticity, including habituation, and provide a promising model system to which we can apply the design of invertebrate behavior based screens to reveal the genetic mechanisms critical for cognitive function in vertebrates. Using zebrafish larvae, we performed the first genetic screen for vertebrate learning mutants and identified 14 mutants with reduced habituation. Whole genome sequencing of a subset of mutants identified a premature stop codon in the vertebrate specific *pregnancy associated plasma protein-a (papp-a)* gene. PAPP-A has been shown to enhance insulin-like growth factor (IGF) availability, and while IGF-receptor signaling plays roles in neural circuit assembly, plasticity and memory formation, the precise roles of papp-a in the nervous system and how it regulates habituation are unknown. Thus, papp-a provides a unique entry point to decipher the neural circuits underlying vertebrate habituation. To dissect papp-a's role, we have combined genetic and pharmacological approaches, neural circuit analysis, and various behavioral assays. We will present our results suggesting that papp-a controls habituation by acutely regulating canonical IGF-receptor signaling.

44. Postsynaptic Neurobeachin is required for electrical and chemical synaptogenesis. ***Adam Miller***, *Lisa Voelker, Arish Shah, Cecilia Moens.* Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.
Neural circuit organization underlies all of behavior. In order to make circuits, neurons must direct their processes to the correct location, recognize their synaptic partners, and transport proteins to the sites of synaptogenesis. Synapses can be chemical, where signals are transmitted via neurotransmitter release and reception, or electrical, where signals pass directly through gap junctions. To identify genes required for synaptogenesis we performed a forward genetic screen using the zebrafish Mauthner (M) neural circuit. M mediates a stereotyped startle response to threatening acoustic stimuli using a combination of electrical and chemical synapses on both its dendrites and axon. The screen identified a mutant, *disconnect4* (*dis4*), which causes reductions in the localization of electrical (Cx36, ZO-1) and inhibitory chemical (glycine receptor, gephyrin) synaptic proteins at M dendritic and axonal synapses. The loss of synaptic proteins correlates with functional deficits where mutants fail to initiate startle responses to auditory stimuli, have defects in balance, and are less responsive than wildtype animals. RNAseq-based mapping and cloning of *dis4* mutants identified a nonsense mutation in the autism-associated gene *neurobeachin* (*nbeaa*) and non-complementation with TALEN induced deletions confirmed its causal role. Nbea is a

highly conserved, large, multidomain protein expressed throughout the nervous system where it localizes to tubulovesicular membranes found from the Golgi to the synapse. Genetic and biochemical analysis in mouse suggests that Nbea plays a role regulating the transport of cargo at GABAergic and Glutamatergic chemical synapses. Whether Nbea acts on the pre- or postsynaptic side of the synapse, or both, remains unknown. We analyzed chimeric animals in which the pre- or postsynaptic neuron was *nbea* mutant while its partner was wildtype and found that Nbea is necessary and sufficient in the postsynaptic neuron for electrical and chemical synaptogenesis. We conclude the Nbea acts postsynaptically to broadly control synapse formation and its localization to the endomembrane system suggests it may control the dendritic transport of synaptic cargo.

45. Genetic dissection of zebrafish circadian behaviors by TALEN and CRISPR/Cas9. ***Han Wang***, *Jian Huang, Shuqing Zhang, Yanqing Zhang.* Center for Circadian Clocks, Soochow University, Suzhou, Jiangsu, China.
Zebrafish display robust daily changes in physiology and behavior. However, scarce of zebrafish genetic circadian mutants has hampered further development of the zebrafish circadian field. Using retroviral insertional mutagenesis, TALEN or CRISPR/Cas9, we have generated zebrafish mutants for four zebrafish *period* (*per*) genes: *per1a*, *per1b*, *per2* and *per3*. Here we report characterization of these zebrafish *per* mutants. Behavioral assays showed that under light/dark (LD) condition, *per1b* mutant larvae were approximately three times more active than their wild-type counterparts, while *per2* mutant larvae had approximately 30% reduced activity amplitude in comparison with wild types; under constant darkness (DD) condition, locomotor activity of *per1b* mutant larvae was phase advanced by two hours, while locomotor activity of *per2* mutant larvae was phase delayed by two hours. Under DD condition, the expression of *per1a, per2* and *per3* was all significantly increased in *per1b* mutants. These results indicate that Per1b serves as a negative regulator of the three other per genes in the zebrafish circadian system. Under LD condition, *bmal1a* and *annat2* were significantly up-regulated but *per1a, per1b* and *bmal1b* were significantly down-regulated in the *per2* mutant fish. *In vitro* cell transfection assays showed that Per2 not only acts through E-boxes to repress E-Box-containing genes but also acts through RRE-boxes to activate RRE-box-containing genes. We also found that zebrafish *per1b* mutants display hyperactive-, impulsive-, and attention deficit-like behaviors and low levels of dopamine, reminiscent of human Attention Deficit Hyperactivity Disorder (ADHD) patients. The circadian clock contributes to ADHD pathogenesis by regulating the dopamine catabolism genes and likely the number and organization of dopaminergic neurons in the ventral diencephalic posterior tuberculum (PT). Our studies demonstrate that Per2 plays dual roles in zebrafish circadian regulation and disruption of a circadian clock gene elicits ADHD-like syndrome. We also will present our analyses of *per1a* and *per3* mutants as well as double *per* mutants in the conference.

46. Tbx1 controls the morphogenesis of pharyngeal pouch epithelia through mesodermal Wnt11r and Fgf8a. ***Chong Pyo Choe***, *Gage Crump*. Broad CIRM Center, University of Southern California, Los Angeles, CA.
The pharyngeal pouches are a segmental series of epithelial structures that organize the embryonic vertebrate face. In mice and zebrafish carrying mutations in homologs of the DiGeorge Syndrome gene TBX1, a lack of pouches correlates with severe craniofacial defects, yet how Tbx1 controls pouch development remains unclear. By analyzing early markers of developing pouches, we reveal that Tbx1 is required for pouch morphogenesis but not initial segmentation. Especially, despite the near complete loss of morphological pouches in *tbx1* mutants, we still observe segmental *fgf3* expression, as well as segmental Fgf activity as measured by the *dusp6*:dGFP reporter. Next, using mutant and transgenic rescue experiments, we show that Tbx1 functions primarily in the mesoderm to promote the morphogenesis of pouch-forming endoderm through *wnt11r* and *fgf8a* expression. In particular, we find a specific requirement for Tbx1 in inducing expression of both *wnt11r* and *fgf8a* in the facial mesoderm. Consistent with Wnt11r and Fgf8a acting together downstream of Tbx1, compound losses of *wnt11r* and *fgf8a* phenocopy *tbx1* mutant pouch defects, while mesoderm-specific restoration of Wnt11r and Fgf8a rescues *tbx1* mutant pouches. Furthermore, time-lapse imaging reveals that Fgf8a acts as a Wnt11r-dependent chemoattractant for migrating pouch cells. Together, our work reveals that Tbx1 promotes the segmental outpocketing of pouch epithelia by coordinating Wnt-dependent epithelial destabilization of pouch-forming cells with their collective migration towards Fgf8a-expressing mesodermal guideposts.

47. Exploring the zebrafish periderm gene regulatory network downstream of IRF6 reveals a novel human orofacial clefting locus GRHL3. ***Robert A Cornell***[1], *Myriam Peyard*[2], *Gabriel De la Garza*[1], *Jack Schleiffarth*[1], *Greg Bonde*[1], *Dan Wagner*[3], *Juha Kere*[2], *Douglas Houston*[1], *Jefferey Murray*[1], *Tiffany Smith*[1]. 1) Anatomy and Cell Biology, University of Iowa, Iowa City, IA; 2) Karolinska Institue, Stockholm, Sweden; 3) Rice University, Houston, Texas.
Orofacial clefting (OFC) is a common birth defect with syndromic and non-syndromic forms. Mutations in IRF6, encoding transcription factor Interferon Regulatory Factor 6, are found in most patients with Van der Woude syndrome, which includes OFC, and sequence variants near IRF6 are associated with elevated risk for non-syndromic OFC. Despite the success of genome wide association studies, about half of the heritable risk for non-syndromic OFC remains unassigned to any genes. We reasoned that genes in the same regulatory pathway as IRF6 are candidate cleft loci. We have shown that in zebrafish and frog embryos, Irf6 is necessary for differentiation of the embryonic superficial epithelium, or periderm. Here we use microarrays to identify genes that a) are expressed in the zebrafish periderm and b) whose expression is inhibited by a dominant-negative variant of Irf6 (dnIrf6). These methods identify Grainyhead-like 3 (Grhl3), an ancient regulator of the epidermal permeability barrier, as acting downstream of Irf6. In human keratinocytes, IRF6 binds conserved elements near the GRHL3 promoter. Intriguingly, forced grhl3 expression restores periderm markers in both zebrafish injected with dnIrf6 and frog embryos depleted of Irf6. We sequenced GRHL3 in 30 Van der Woude syndrome patients lacking mutations in IRF6 and found missense mutations in 7 such patients. In zebrafish, we confirmed that patient-derived variants of GRHL3 have dominant negative function. In summary, Grhl3 is a key effector of Irf6 in periderm differentiation, and dissection of the periderm gene regulatory network has helped identify a novel gene underlying orofacial clefting.

48. A Wnt-BMP signaling network balances growth and differentiation during zebrafish bone regeneration. ***Scott Stewart***[1], *Alan Gomez*[1,2], *Benjamin Armstrong*[1], *Astra Henner*[1], *Kryn Stankunas*[1,2]. 1) Institute of Molecular Biology, University of Oregon, Eugene, OR; 2) Department of Biology, University of Oregon, Eugene, OR.
In contrast to mammals, zebrafish bones completely regenerate following their damage or loss. An understanding of this amazingly robust process could provide insights towards new therapies for human bone injuries. Recent studies demonstrate that bone regeneration in zebrafish fins is mediated by lineage-restricted osteoblasts that subsequently produce replacement bone. However, the molecular mechanisms that control the osteoblast regenerative program from its initiation through re-ossification are poorly understood. We find that, upon fin amputation, a pool of proliferative twist2+/Runx2+ pre-osteoblasts is generated by an epithelial to mesenchymal transformation (EMT) of mature osteoblasts. The Wnt/b-catenin pathway sustains these Runx2+ progenitors, which concentrate near the distal tip of the regenerating fin mesenchyme. Bone Morphogenetic Protein (BMP) subsequently promotes osteoblast re-differentiation by activating sp7 expression. Further, BMP constrains Wnt activity by inducing the Dickkopf-related Wnt antagonist, dkk1b. This simple feedback network maintains the requisite balance between bone growth and differentiation until regeneration is complete. While BMP-dependent osteoblast maturation is lineage intrinsic, the Wnt ligands needed to sustain pre-osteoblasts are produced by neighboring non-osteoblast cells that comprise a “stem cell” niche. Our current efforts focus on identifying the cell lineage that comprises the niche and the mechanisms that promote niche formation upon fin amputation. These studies will establish a framework for generating mammalian cells that perform analogous functions and therefore could be used to improve osteogenesis during fracture healing.

49. Zebrafish mutants with craniofacial malformations reveal novel facets of suture formation. ***Erika Kague***[1], *Garrett Asselin*[2], *Craig Albertson*[2], *Shannon Fisher*[1]. 1) Cell and Developmental Biology , University of Pennsylvania, Philadelphia, PA; 2) Department of Biology, University of Massachusetts, Amherst.
During skull growth, the bones of the cranial vault overlap at the sutures. Suture architecture is highly conserved and includes an orderly progression of osteogenesis inward from the edges of the bones, or osteogenic fronts (OFs). Identified human mutations suggest that changes in the rate of osteogenesis disrupt sutures in predictable ways, either leading to open sutures or their premature fusion. We have characterized two zebrafish mutations that disrupt suture architecture and prevent the normal overlap, but do not fit into this accepted framework. The first of these is in the transcription factor sp7, required for normal osteoblast differentiation. Mutants have deformed and fractured bones and develop scoliosis. Morphometric analysis sorts sp7 mutants into a unique group with midface hypoplasia, protruding jaw and a bent parasphenoid. Through sequential live imaging of transgenic fish we observe progressive formation of extra sutures and additional cranial bones from ectopic ossification sites in mutants. Histologically cranial bones in sp7 mutants are thinner, with impaired mineralization and rounded OFs separated by mesenchymal cells. Early osteoblasts are highly proliferative and not limited to the OFs, as in WT. We identified a mutation in the toth gene, as part of an ongoing screen for craniofacial mutants. Mutants in toth have normal axial skeleton, although the bone appears porous and strongly Alizarin red stained. Mutants display brachycephaly, domed skull and shorter face, although they have normally patterned sutures. Similar to sp7 mutants they form non-overlapping sutures, although histologically the bone appears overmineralized. We are currently examining osteogenesis and proliferation in toth mutants, and working to molecularly identify the gene. Mutations in sp7 and toth respectively delay and accelerate differentiation, but neither has the predicted effects on suture architecture or skull development. We have developed a revised model of regulation at the sutures, incorporating the new aspects of suture biology revealed by our analysis of these mutants.

50. Osteocyte-dependent and -independent osteoclast activity as a major mediator of RA-induced skull fragmentation. ***Shirine Jeradi***, *Matthias Hammerschmidt.* University of Cologne, Germany.
Over exposure to Retinoic Acid (RA) has long been associated with increased bone fragility in both humans and animal models, and an elevated RA level caused by a loss-of-function mutation in the RA metabolizing enzyme CYP26B1 leads to the formation of fragmented calvarial plates in human fetuses. Despite the numerous in vivo and in vitro studies that address the effect of elevated RA levels on skeletogenesis, it remains unclear which cell population mediates the bone fragility phenotype, and /or compromised bone formation. In this study, we used the zebrafish as a model to understand the effect of RA on bone development and maintenance. We show that RA has a negative effect on mature osteoblast activity and causes reduced growth of calvarial bones, via promoting a premature differentiation of osteoblasts towards an osteocytic fate. We also have evidence that RA induces active bone resorption, due to an increase in osteoclast activity, resulting in development of holes in the skull. In the absence of osteoclasts, RA treatment does not have any effect on bone resorption. However, RA also fails to induce holes in the fish skull after the ablation of the mature osteoblasts, pointing to a requirement of both osteoblasts and osteoclasts to induce the observed phenotype. Recent studies have shown that, in vivo, osteocytes, as compared to osteoblasts, have an increased ability to recruit osteoclasts to the site of bone resorption. Therefore, we propose a model in which the increased osteoclasts recruitment is promoted by the premature differentiation of osteoblasts to osteocytes mediated by increased RA. However, we also have indications that the osteoclasts themselves show a mild direct response to RA, which suggests that osteoclasts are also a primary mediator of RA signaling. In order to determine the cell populations directly responding to RA treatment, we are currently establishing transgenic lines expressing an inducible dominant negative RA receptor in either osteoblasts or osteoclasts. These lines will enable us to specifically block RA signaling in distinct cell populations, allowing us to dissect which cell(s) directly respond to the RA signal, and mediate the development of the holes in the skull.

51. Notch Signaling Enhances Self-renewal in Embryonal Rhabdomyosarcoma. ***Myron S. Ignatius***[1], *Eleanor Chen*[1], *Karin McCarthy*[1], *Riadh Lobbardi*[1], *Corinne Linardic*[2], *Charles Keller*[3], *David Langenau*[1]. 1) Massachusetts General Hospital and Harvard Medical School, Boston, MA; 2) Duke University Medical Center, Durham, NC; 3) Oregon Health & Science University, Portland, OR.
Embryonal rhabdomyosarcoma (ERMS) is a devastating pediatric muscle cancer. Work from our group has identified the tumor-propagating cell (TPC) in a transgenic zebrafish model of kRASG12D-induced ERMS that is responsible for driving continued tumor

growth and relapse. The TPC is molecularly similar to an activated muscle satellite-cell and expresses *myf5*, *c-met*, and *m-cadherin*. Building on these observations, we have identified the Notch pathway as a potent enhancer of ERMS self-renewal and TPC number. Specifically, TPCs are increased 10-fold in kRASG12D expressing ERMS that co-express activated intracellular Notch1 (ICN1) (n>4 ERMS/genotype). Moreover, ICN1 expressing ERMS exhibit a 3-fold expansion of relapse-driving myf5-GFP+/mylz2-mCherry-negative cell population. Unexpectedly, cell transplantation and cell lineage tracing experiments revealed that Notch pathway activation conferred tumor-propagating ability to the myf5-GFP+/mylz2-mCherry+ mid-differentiated ERMS cells - a population of cells previously shown to lack self-renewal capacity. To validate our findings in human ERMS, a role for NOTCH in regulating self-renewal and differentiation in human disease was examined. Human ERMS cells that expressed activated NOTCH1 had elevated sphere-colony formation, a surrogate for self-renewal *in vitro*. By contrast, shRNA knockdown of NOTCH1 resulted in decreased sphere-colony formation and robust terminal differentiation of ERMS cells into late-stage, MYOSIN-expressing myoblasts. Moreover, we identified that NOTCH1 regulated *SNAI1* expression is required for self-renewal and differentiation of human ERMS. SNAI1, a transcriptional repressor, competes with MYOD for binding to muscle differentiation gene targets blocking differentiation. Taken together, our data indicate that Notch signaling is an important modifier of human ERMS acting through *SNAI1* to regulate both TPC self-renewal and differentiation. Our data suggests that Notch pathway inhibition will have provide benefit to a large subset of patients with metastatic or relapsed ERMS.

52. Neuronal Signaling is Necessary for Zebrafish Appendage Regeneration. ***Timothy A Petrie***[1,2], *Nicholas S Strand*[1,2], *Anthony Recidoro*, *Aspen Gutgsell*[3], *Ronald Y. Kwon*, *Randall T Moon*[1,2]. 1) Howard Hughes Medical Institute; 2) Department of Pharmacology, Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA; 3) HHMI EXROP Scholar Program.
Zebrafish possess a robust capacity to regenerate amputated fins. Classical denervation studies suggest that nerve presence contributes significantly to level of appendage regeneration, but, importantly, regeneration does not depend on neurotransmitter (acetylcholine) release or signaling. Using multiple, innovative approaches to disrupt Zebrafish neuronal signaling (without physical denervation) in the caudal fin prior to fin amputation, we show that nerve signaling modulates the fin regeneration response and is necessary for full tissue regeneration. We perturbed signaling independently via three techniques: 1) spinal cord resection (SCT), which inhibited neural signaling to posterior appendages, 2) Acetylcholine Transferase morpholino injection (ChAT) into the caudal peduncle, which reduced acetylcholine release, and 3) injection of Botulin Toxin B (a clostridial neurotoxin that inhibits synaptic fusion, BOTOX) into the caudal peduncle, inducing rapid, dose-dependent, and transient muscle paralysis. All three models of signaling disruption significantly inhibited the degree of tissue regeneration (>90% inhibition, SCT; >50%, ChAT; >60%, BOTOX). Moreover, SCT treatment reduced fin blastema formation, size, and proliferative capacity. Moreover, all treatments significantly reduced expression levels of regeneration-associated genes, yet augmented inflammation. Interestingly, SCT and ChAT treatments significantly reduced the numbers of cells undergoing Wnt/b catenin signaling, a pathway essential for fin regeneration. Importantly, persistent overexpression of a Wnt ligand (after SCT treatment) using a heat-shock inducible transgenic fish line (hsWnt8:GFP) partially rescued proliferation and regeneration, increasing total tissue regrowth 4 fold higher than non-Wnt stimulated animals. Collectively, this data provides cogent evidence that neuronal signaling, in addition to physical nerve presence, is required for optimal Zebrafish appendage regeneration, and that modulating Wnt/b catenin signaling may partially override the deleterious effects of neuronal signaling disruption.

53. Imaging *live* Cell Dynamics of the Muscle Progenitor Cell Niche During Development. ***P. D. Nguyen***, *P. D. Currie.* Monash University, Melbourne, Clayton, Australia.
Vertebrate muscle is derived from the dermomyotome. In the zebrafish, the equivalent structure is known as the external cell layer (ECL). It is an environment where progenitors are provided for muscle growth both during and after embryogenesis. Consequently, the balance between self-renewal and supplying progenitors must be highly controlled. It remains to be elucidated how this balance is achieved within the ECL. We used *live* time-lapse imaging to show that the establishment of the ECL at the end of somitogenesis (1 day post-fertilization) involves a dynamic and diverse set of proliferation modes. These proliferation modes can be classified according to the orientation of the resulting daughter cells relative to the myosepta. We also observed ECL cells migrating into the myotome to populate an independent pool of highly motile, proliferative cells. The emergence of a second proliferative progenitor pool within the myotome suggests the source of progenitors is not solely restricted to the ECL. By observing *live* cell dynamics of the stem cell niche during myogenesis, we are able to examine the dynamic behaviors of muscle progenitors, and aid in understanding how progenitors maintain their numbers during development.

54. Spatial regionalization and heterochrony in the formation of adult pallial neural stem cells. *Lara Dirian*[1], *Sonya Galant*[1], *Marion Coolen*[1], *Wenbiao Chen*[2], *Sébastien Bedu*[1], *Corinne Houart*[3], *Laure Bally-Cuif*[1], ***Isabelle Foucher***[1]. 1) Institute of Neurobiology A. Fessard, Laboratory of Neurobiology and Development, CNRS UPR3294, Team Zebrafish Neurogenetics, Avenue de la Terrasse, bldg 5, F-91198 Gif-sur-Yvette, France; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, 2213 Garland Ave, Nashville, TN, 37232, USA; 3) Medical Research Council Centre for Developmental Neurobiology, King's College London, London SE1 1UL, UK.
Little is known on the embryonic origin and related heterogeneity of adult neural stem cells (aNSCs) in the vertebrate brain. We use conditional genetic tracing, activated in a global or mosaic fashion by cell type-specific promoters or focal laser uncaging, coupled with gene expression analyses and Notch invalidations, to address this issue in the zebrafish adult telencephalon. We report that the germinal zone of the adult pallium originates from two distinct subtypes of embryonic progenitors and integrates two modes of aNSC formation. Dorso-medial aNSCs derive from the amplification of actively neurogenic radial glia of the embryonic telencephalon. On the contrary, the lateral aNSC population is formed by step-wise addition at the pallial edge from a discrete neuroepithelial progenitor pool of the posterior telencephalic roof, activated at post-embryonic stages and persisting life-long. This dual origin of the pallial germinal zone allows the temporally organised building of pallial territories as a patchwork of juxtaposed compartments.

55. Cardiac outflow tract signals control the regenerative potential of the zebrafish epicardium. ***Jinhu Wang***, *Jingli Cao, Amy L. Dickson, Kenneth D. Poss.* Department of Cell Biology and Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710, USA.
Upon cardiac injury, a mesothelial structure enveloping the heart called the epicardium is activated to proliferate and induce embryonic markers. Epicardial cells created by this expansion cover and integrate into the injury, providing paracrine signals for cardiac muscle cell survival or division, differentiating into perivascular components and possibly other cell types, and modulating inflammation. Despite intense recent interest in the epicardium, little is known of how this tissue is maintained and stimulated by injury in the adult context. Here, we used a genetic ablation system to specifically lesion a large proportion of epicardial cells in adult zebrafish. This treatment blocked the capacity for cardiac muscle regeneration, which is normally high in zebrafish. Moreover, in response to this injury, epicardial tissue quickly regenerated from spared cells in a base to apex wave upon the ventricular surface. By extirpation and tissue recombination procedures in explanted hearts cultured ex vivo, we found that cardiac outflow tract is both necessary and sufficient to direct regeneration of ventricular epicardium. Chemical screening revealed that Hedgehog signaling is required for epicardial regeneration, whereas Shh-soaked beads could restore epicardial regeneration ex vivo after removal of outflow tract tissue. Our experiments indicate that the epicardium has a high capacity to regenerate, and that this regeneration is directed by Hedgehog signals from the cardiac outflow tract, findings with implications for developing the epicardium as a target for cardiac repair.

56. Non-toxic, zebrafish-optimized optogenetic gene expression system with large dynamic range and rapid kinetics. ***A Reade***[1,2], *DY Stainier*[1,2,3], *OD Weiner*[1,2], *S Woo*[1]. 1) Cardiovascular Research Institute; 2) Dept of Biochemistry and Biophysics, UCSF, San Francisco, CA; 3) Max Planck Institute for Heart and Lung Research, Dev Genetics, Bad Nauheim, Germany.
Current inducible expression systems in zebrafish rely on administration of small molecule compounds or activation of heat shock promoters, which are limited in fine-grained spatial and temporal control. An optogenetic gene expression system would allow for precise induction of transcription both temporally and spatially. Several useful optogenetic systems have been developed, but so far, these systems are not ideally suited for use in zebrafish. In order to be truly useful for studying zebrafish development, a system would need to be: genetically encoded, not require complicated optics or exogenous small molecules, have a large range of induction, be reversible with fairly quick kinetics and, most importantly, have little to no toxicity. Recently, two systems, LightOn and EL222, showed the promise to fulfill all of these qualifications if optimized for zebrafish use. Both systems consist of an engineered bacterial light-oxygen-voltage protein fused to a transactivating domain that dimerizes and induces transcription when illuminated with blue light. We have tested both systems in zebrafish and found them both easily expressed and able to strongly induce expression of fluorescent reporter constructs. Although toxicity of LightOn and EL222's transcription factors in zebrafish embryos is minimal at lower concentrations, we observed significant toxicity at higher concentrations. Studies have shown that high levels of strong transactivating domains are toxic for zebrafish development. We therefore replaced the VP16 or p65 domain of EL222 and LightOn, respectively, with KalTA4, a zebrafish optimized transactivating domain. This switch removed all toxicity up to 150pg of injected mRNA/embryo. We then tested each system and found only the reengineered EL222 system, TAEL, to still be functional. We have made stable transgenic lines to: 1. fully characterize the TAEL system 2. control the expression of the master transcription factor Sox32 and 3. inducibly express diphtheria toxin to serve as a highly sensitive cell ablation system.

57. On the role of mechanical strains on dynamic cellular shape changes in developing zebrafish heart. ***Vikas Trivedi***[1], *Le Trinh*[2], *Thai Truong*[2], *Scott Fraser*[2]. 1) Bioengineering, California Institute of Technology, Pasadena, USA; 2) Department of Molecular Biology, University of Southern California, Los Angeles, USA.
The developing heart is a highly dynamic organ generating multiple types of forces, at different length and time scales, crucial for its morphogenesis. In a multicellular setting, the field of anisotropic physical forces represents a source of information for the cells to know their relative position and thus guide cellular shape and differentiation. However the exact spatio-temporal relationship between the forces, cellular changes and morphogenesis in the context of cardiac development is not clearly understood, largely due to the inability to image its intrinsic dynamics at high resolution. To meet these challenges, we have employed systematic confocal imaging combined with 2-photon light sheet microscopy (2p-SPIM) to document the cell shape changes that occur over the developmental time scale in the embryonic zebrafish heart. The dynamic imaging enables us to estimate the strain that the myocardium experiences as it differentiates into atrium and ventricle undergoing morphogenesis from a cylindrical tube to a 100-micron length scale, two chambered organ, moving over tens of microns amplitude thereby posing challenges for sub-cellular resolution imaging. Our confocal imaging shows that not only the average cell size increases with development, but also the variability increases indicating an increase in tissue heterogeneity over time. Correspondingly the strains inferred from 2p-SPIM imaging of myocardium also show heterogeneity along the length of the heart tube over time. Mutations in the contractile machinery of the myocardial cells enable us to establish the link between mechanical strains and cell shape essential for the maintenance of the tissue geometry. These results provide the groundwork needed to start deciphering the processes linking dynamic changes in cellular shapes, gene expression, and cellular physical properties participate, in the development of the vertebrate heart.

58. Controlling apico-basal polarity within a zebrafish embryo. ***Clare E. Buckley***[1], *Anna Reade*[2], *Casper C. Hoogenraad*[3], *Orion D. Weiner*[2], *Jon D. W. Clarke*[1]. 1) MRC Centre for Developmental Neurobiology, King's College London, London, United Kingdom; 2) Cardiovascular Research institute, University of California-San Francisco, San Francisco, California, USA; 3) Faculty of Science, Utrecht University, Utrecht, The Netherlands.
We have recently published a novel mechanism of cell polarisation and lumen formation within the zebrafish hindbrain: zebrafish neuroepithelial cells are able to detect the point where they intersect the midline of the developing organ primordium and traffic apical proteins to that point via a microtubule dependent mechanism (Buckley et al., 2013). We put forward the hypothesis that a self-reinforcing

loop exists between protein partitioning defective 3 (Pard3) and centrosome interactions in order to specify the precise location of the neural rod midline. We would now like to directly test the relative importance of these components in establishing and maintaining neuroepithelial polarity and lumen formation during zebrafish hindbrain neural tube morphogenesis. In order to do this at a specific location within individual cells, without knocking out proteins globally, we need to develop methods to artificially move polarity proteins within individual cells in the living zebrafish embryo in a spatially and temporally controlled manner, in combination with in vivo imaging. We are attempting this in two ways. First, we have validated the use of the FKBP-rapalog-FRB heterodimerisation system *in vivo*. Addition of the rapamycin analogue rapalog enables the binding of the FKBP domain to FRB. This induced heterodimerisation can be used to experimentally bind a protein of interest as cargo to a motor protein that will then relocalise the protein (Kapitein et al, 2010a; Kapitein et al, 2010b). Second, in an attempt to gain further temporal and spatial control of protein localisation, as well as enabling reversal and titration of binding, we are developing the use of the red light-inducible Phytochrome (PhyB-PIF) system *in vivo*. The Arabidopsis phytochrome B (PhyB) protein is induced to bind to a transcription factor phytochrome interaction factor (PIF) under red light. This binding is reversed under far-red light (Levskaya et al, 2009; Yang et al, 2013).

59. Zebratrack: Shining light on vertebrate behavior. *Pierre Martineau, **Philippe Mourrain***. Psychiatry and Behavioral Sci, Stanford University, Stanford, CA.
The complexity of the brain makes the analysis and discovery of the underlying neural circuitry of behavior difficult. Optogenetics offers a novel and powerful avenue to interrogate circuit function. When applied to a transparent animal, optogenetics can realize a formidable potential to functionally probe the individual circuits that constitute a brain and hence identify circuits disrupted in behavioral disorders. Zebrafish (Danio rerio) is the major vertebrate genetic model available today that develops transparently. Its optical clarity makes it ideal for experimenters to employ the revolutionary optogenetic tools, that allow precise control of neuronal activity in a non-invasive regime, by simply shining light(s) on neurons expressing light-gated ion channels. Importantly, zebrafish share similar but more compact nervous system architecture with mammals, where important circuits for behavioral regulation are conserved. Thus zebrafish has great and unexploited potential for circuit analysis and discovery using optogenetic methods. An intriguing hurdle to this endeavour is that most transparent zebrafish strains suitable for optogenetics are so optically clear that no current tracking strategy can efficiently detect them, which is crucial to support the combination of state-of-the-art optogenetic approaches and parallel behavioral recording of groups of free-swimming zebrafish. Also, to date, the pace of progress has been hindered by the fact that currently available monitoring tools are expensive, have limited precision, and require isolating individual fish in separate cells. To solve this challenge, we have developed Zebratrack an innovative method based on darkfield illumination and low frame rate image capture that can reveal and track individual transparent zebrafish within a group while they are simultaneously stimulated for optogenetics. Using movies showing behavioral examples we will present what we believe to be the first method supporting multitracking of free swimming zebrafish and optogenetic control of behaviors.

60. Genome-wide RNA tomography in the zebrafish embryo. *Jan-Philipp Junker[1], **Emily S. Noël**[1], Eugene Berezikov[2], Jeroen Bakkers[1], Alexander van Oudenaarden[1]*. 1) Hubrecht Institute, Utrecht, Netherlands; 2) ERIBA, Groningen, Netherlands.
Progressing our understanding of embryonic development relies heavily on the identification of novel pathways or interactions required for specific processes. While microarrays, and more recently RNA sequencing, has allowed us to investigate the transcriptome of an embryo or tissue, and in situ hybridization provides us with spatial information about gene expression, producing large-scale transcriptome information in a spatially resolved manner has previously been limited.We have developed a technique termed genome-wide RNA tomography where we combine traditional histology techniques with low-input RNA sequencing and mathematical 3D image reconstruction to generate a 3D atlas of gene expression in the zebrafish embryo. In this technique we cryosection multiple embryos along 3 axes at high resolution, extract RNA and use a modified single-cell RNA-seq protocol to amplify and generate libraries for 3'UTR sequencing. We then map the transcriptome datasets from each axis back onto a 3D reconstruction of a reference embryo, providing accurate spatial information about gene expression at a chosen timepoint. By taking genes of interest for a tissue or process, we can search for genes (known or unknown) that are coexpressed in the region of interest. Currently we have generated 3D atlases of shield stage, 10 somite stage and 15 somite stage embryos. Analysis of mapped gene expression of reference genes (for example in the organizer at shield stage) reveals that our 3D mapping accurately places gene expression within a 3D mask of an embryo. Using traditional in situ hybridization techniques, we have furthermore been able to validate novel expression patterns predicted by RNA tomography, confirming that our method can identify spatial variations in levels of gene expression.This technique provides a powerful resource to gain accurate spatial information about the transcriptome in the embryo or tissue of interest at any given timepoint. Advantages of this technique include the ability to investigate spatial differences in gene expression, to pick up low levels of gene expression that are undetectable by standard in situ techniques, and to identify novel candidates and interactors required for embryonic development.

61. Cadherin 2 Orchestrates Tissue Self-Assembly Through Two Opposing and Spatially Non-Overlapping Mechanisms. ***Patrick McMillen**, Dörthe Jülich, Scott Holley.* Yale University, MCDB, New Haven, CT.
Patterned configurations of adherent cells and Extracellular Matrix (ECM) underlie the topological and mechanical diversity of animal tissues. We have previously shown that vertebrate trunk elongation is propelled via cell-Fibronectin mechanics within the paraxial mesoderm. This tissue self-assembles from motile mesodermal progenitors in the posterior tailbud. Here, we examine how cell-cell and cell-ECM adhesion are integrated during this tissue assembly. We specifically examined Cadherin 2 (Cdh2) depletion by itself and in conjunction with depletion of the primary Fibronectin receptor Integrin a5. Paradoxically, this analysis suggests that Cdh2 both stimulates and inhibits ECM fibrillogenesis. We resolve this apparent contradiction via genetic mosaics and demonstrate that Cdh2 restricts ECM formation to tissue boundaries through two distinct mechanisms: an inhibitory b-Catenin dependent mechanism and a stimulatory b-Catenin independent mechanism. These data suggests Cdh2 as a key node that represses ectopic ECM among adherent cells within a tissue

and promotes ECM fibrillogenesis along tissue boundaries. As Fibronectin fibrillogenesis is a mechanically driven process dependent upon actomyosin contractility, we hypothesize that Cdh2 mediates its stimulatory role by localizing the force exerted by cells on the ECM to tissue boundaries. To test this hypothesis we generate a novel mechanical force sensor that detects high magnitude tension between cells and the Fibronectin matrix. Uniquely, our probe relies upon ratiometric imaging and the mechanical stability of GFP rather than FRET. The force sensor highlights known sites of Fibronectin assembly in a manner dependent upon Integrin a5 function and Integrin binding. We are currently working to apply this sensor to measure the role of Cdh2 in organizing the mechanical environment of the developing embryo.

62. E-cadherin-dependent cell adhesion regulates cell migration pattern in the zebrafish lens epithelium. ***Toshiaki Mochizuki****, Yi-Jyun Luo, Shohei Suzuki, Ichiro Masai.* OIST, Okinawa, Japan.
The vertebrate lens consists of anterior proliferative lens epithelium and posterior lens fiber core. Lens epithelial cells differentiate into lens fiber cells at the peripheral margin of the lens epithelium called the equator. Here we used transgenic lines expressing fluorescent protein-tagged Geminin and Histone to visualize cell cycle progression and cell migration. In our time-lapse scanning of lens epithelium from 36 to 48 hpf, a majority of cells migrated along the longitudinal axis in the most anterior region, but their migratory direction was shifted from longitudinal to circumferential, and most of cells migrated circumferentially in the peripheral region. Furthermore, migratory direction was abruptly changed to longitudinally in the transition zone posterior to the equator. In addition, migration speed was increased according as cells move towards posteriorly. These observations suggest that cell migration is spatially regulated in the lens epithelium. Next we examined cell intercalation and cell division. Cell intercalation was rare when cell division was not accompanied. In 12 hours, more than 50% of cells did not undergo cell division. Such non-dividing cells did not intermingle with mitosis-experienced cells but form 5-10 cell clusters, whose relative positions were not rearranged. On the other hand, mitosis-experienced cells formed clusters, in which cell divisions promoted surrounding cell movements and rearrangement. These data suggest that cell division is a major driving force for cell migration in the lens epithelium. To understand its molecular mechanism, we focused on E-cadherin, which is expressed in the lens epithelium. In *E-cadherin* mutant, *half baked* (*hab*), cell migration was biased more longitudinally, migration speed was higher, and inter space between neighboring nuclei was wider than in wild type, suggesting that E-cadherin keeps cells within the lens epithelium. Although non-dividing cells and mitosis-experienced cells were clustered in *hab* mutant, cell division and surrounding cell movement was uncoordinated. These observations suggest that E-cadherin-dependent cell adhesion is required for spatial coordination between cell division and cell migration in the lens epithelium.

63. Epithelial flow into the optic cup facilitated by suppression of BMP drives eye morphogenesis. ***Stephan Heermann***[1,2]*, Lucas Schütz*[1]*, Steffen Lemke*[1]*, Kerstin Krieglstein*[2]*, Jochen Wittbrodt*[1]. 1) COS Heidelberg, University Heidelberg, Heidelberg; 2) Anatomy and Cell Biology Freiburg, University Freiburg, Freiburg.
The bi-layered vertebrate optic vesicles are formed by the bilateral evagination of the late prosencephalon, a process that in teleosts is driven by single cell migration. The transition of the oval optic vesicle to a hemispheric bi-layered optic cup involves major morphological transformations. In the classical view, the lens-averted epithelium of the optic vesicle differentiates into the retinal pigmented epithelium (RPE), while the lens-facing epithelium gives rise to the neuroretina, which is subsequently bending around the developing lens. This neuroepithelial bending is driven by the basal constriction of lens-facing retinal progenitor cells (RPC), which ultimately reduces the space an individual RPC is demanding. However, at the same time we find a 4.7 fold increase of the basal surface area. We show that the lens-averted epithelium in fact functions as the cell reservoir for the forming neuroretina. Epithelial flow from the lens-averted into the lens-facing domain is driving the transformation from optic vesicle to optic cup. We show that this flow is modulated by BMP activity. Expression of BMP in retinal progenitor cells results in the disruption of epithelial flow during optic cup formation. This inhibition leads to a persisting neuroretina in the domain of the RPE and ultimately results in coloboma. Our data highlight the key contribution of epithelial flow to optic cup morphogenesis. This has far reaching implications for retinal patterning and the origin and establishment of the retinal stem cell niche.

64. Kremen1-mediated modulation of Wnt signaling regulates progenitor cell behavior in the lateral line. ***Hillary F McGraw****, Alex V Nechiporuk.* Cell & Developmental Biology, Oregon Heath & Science University, Portland, OR.
Canonical Wnt signaling plays critical roles during development and disease. However, how Wnt signaling is modulated in different in vivo contexts is not well understood. Here, we investigate modulation of Wnt signaling in the posterior lateral line primordium (pLLp), a cohort of ~100 cells that collectively migrate along the trunk of the zebrafish embryo. The pLLp is comprised of proliferative progenitor cells and organized epithelial cells that will form the mechanosensory organs of the posterior lateral line. Wnt signaling is active in the leading progenitor region of the pLLp and restricted from the trailing zone through expression of the secreted Wnt inhibitors *dkk1b* and *dkk2*. We have identified a mutant zebrafish strain, *kremen1*^{nl10}, which carries a mutation in the kremen1 gene, a non-obligate co-receptor for the Dkk family of proteins. Previous studies showed that Kremen1 negatively regulated Wnt signaling by facilitating internalization of the Dkk-Kremen1-Lrp5/6 complex. Interestingly, we found the disruption of Kremen1 in the pLLp exhibited molecular and cellular phenotypes associated with the loss rather then overactivation of Wnt signaling, including downregulation of Wnt target genes, loss of proliferation in presumptive progenitor cells and increased cell death. Transplantation of wild-type cells into the mutant primordia failed to rescue the *kremen1*^{nl10} phenotype, revealing that the effects of Kremen1 loss are cell non-autonomous; similar phenotype resulted from an ectopic activation of Dkk1b. Inhibition of *dkk1b/dkk2* function partially rescues migration of the pLLP. Based on our data, we propose that Kremen1 modulates Wnt activity by restricting a range of secreted Dkk proteins during collective cell migration of the pLLp, revealing a previously unrecognized mechanism for regulation of canonical Wnt signaling.

65. *Schachbrett*, a zebrafish mutant displaying disrupted stripes, encodes for Tjp1a (ZO-1.1). ***Andrey Fadeev***, *Jana Krauss, Hans-Georg Frohnhöfer, Christiane Nüsslein-Volhard.* MPI for Developmental Biology, Tuebingen, Germany.
Zebrafish owes its name to a pattern of dark stripes and light interstripes, which develops during metamorphosis replacing a larval pattern. The stripes are formed by black melanophores and reflecting iridophores whereas interstripes are formed by yellow xanthophores and dense iridophores. The processes ruling pigment cell migration and positioning in the skin during metamorphosis remain largely unknown and existing models are frequently controversial. Mutagenesis screens have identified a number of genes influencing distinct aspects of stripe pattern formation. We are interested in mutants displaying various levels of stripe disruption, from interruptions to a complete dispersal of melanophores. In this study we have characterized *schachbrett* (German word for chessboard), a mutant displaying interruptions of the stripes by dense iridophores and xanthophores of the interstripe. The *schachbrett* mutant has a premature stop codon in a gene coding Tight Junction Protein 1a. Subsequently several new alleles with the same phenotype were identified, all of which had premature stops in *tjp1a*. Immunohistochemical analysis with antisera raised against two distinct parts of Tjp1a, allowed to detect expression in dense iridophores but no other pigment cell types. In wildtype, after establishment of the first interstripe, iridophores disperse as a loose net along the dorso-ventral axis to contribute to loose iridophores of the dark stripes and new interstripes. Time-lapse imaging of metamorphic animals showed that the formation of the first interstripe in *schachbrett* is normal, but later iridophores invade the developing dark stripes as dense sheets at multiple points along the anterior-posterior axis, changing melanophore dynamics and leading to interruptions of the stripes. While several transmembrane proteins, such as connexins and ion channels, were shown to affect the patterning, their intracellular partners remain unidentified. Since Tjp1a is a scaffold protein which provides an interaction platform for membrane and cytoskeletal proteins, we postulate that the Tjp1 could facilitate the intracellular arm of cell-cell communication downstream of the identified proteins involved in pattern formation.

66. Modeling and preventing anthracycline and tyrosine kinase inhibitor induced cardiotoxicity in Zebrafish. ***Victoria L. Bentley***[1], *Chansey J. Veinotte*[2], *Yan Liu*[3], *Randall T. Peterson*[3], *Graham Dellaire*[1,4], *Jason N. Berman*[2,5]. 1) Pathology, Dalhousie University, Halifax, NS, Canada; 2) IWK Health Centre, Halifax, NS, Canada; 3) Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 4) Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada; 5) Pediatrics and Microbiology & Immunology Dalhousie University, Halifax, NS, Canada.
Anthracyclines are effective anticancer agents for numerous cancers, including leukemia. Unfortunately, cardiotoxicity has emerged as a devastating side effect of anthracyclines, especially in the pediatric context. Targeted anticancer drugs, such as tyrosine kinase inhibitors (TKIs), were developed to minimize off-target effects by specifically targeting tumour cells. However, while effective against Philadelphia positive leukemia, TKIs can cause life-threatening cardiac damage. Thus, it is imperative to develop better animal models of therapy-induced cardiac damage, to efficiently screen for protective drugs that can prevent cardiac damage without impacting the anti-cancer effects of anthracylines or TKIs. We have pioneered an innovative zebrafish human cancer xenotransplantation (XT) model to examine drug-tumor interactions in vivo. Leukemia cell lines were microinjected into 48-hour zebrafish embryos and proliferation rates determined by direct observation in live fish and cell enumeration following embryo dissociation. Engrafted embryos were treated with doxorubicin or TKIs and assessed for cardiac damage and leukemia-drug response. Novel cardioprotective agents (DS1 and DS2) identified in a zebrafish screen were compared to the currently commercially available cardioprotectant, dexrazoxane. Doxorubicin and TKIs have potent anti-cancer activity against leukemia cell lines, but causes cardiac damage in the majority of embryos, with extensive pericardial edema and ventricular collapse. DS1 and DS2 were found to block cardiac damage induced by anthracyclines and TKIs to a greater extent than dexrazoxane, while maintaining tumoricidal activity. Zebrafish human cancer XT permits the rapid evaluation of drug-tumor interactions in vivo while assessing drug toxicity, providing a novel platform for discovery of cardioprotective agents.

67. Novel Renal Progenitor Chemical Screen Identifies Prostaglandins as Modulators of Proximo-Distal Segment Fate Choice During Zebrafish Pronephros Development. ***Shahram J. Poureetezadi***, *Eric K. Donahue, Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46565 USA.
Congenital abnormalities of the kidney and urinary tract (CAKUT) cause renal failure and occur in 1 in 500 live births. There have been significant recent advances in our understanding of kidney ontogeny, but there are many unanswered questions about how different renal cell types arise. The kidney is comprised of segmented functional units called nephrons that consist of a blood filter, tubule and duct. The zebrafish embryo kidney, or pronephros, provides a model to study renal cell type formation due to its high conservation with mammals. Chemical genetics can pinpoint molecules capable of altering physiological processes in the context of the complex humoral environment. Previously, we used small molecules to show that retinoic acid (RA) is essential for proximo-distal segment patterning by altering the balance of renal progenitors. To identify other pathways that modulate renal progenitors, we performed a novel chemical screen. Zebrafish embryos were incubated from 60% epiboly to the 15 somite stage in compounds from the ICCB library (Enzo Life Sciences), fixed at 24 hours post fertilization and then evaluated via whole mount *in situ* hybridization using a riboprobe cocktail that labeled alternating nephron segments. A total of 78/480 compounds (16.25%) caused nephrogenesis phenotypes. Among these, prostaglandins (Pgs) emerged as a family of compounds that consistently affected proximo-distal nephron segmentation. Following Pg treatment, the rostral domain of the proximal convoluted tubule (PCT) was lost. Further, the proximal straight tubule (PST) dramatically expanded at the expense of the distal tubule, such that the distal early (DE) was unchanged but caudally shifted while there was a preferential loss of the distal late (DL) segment. These data suggest a model in which Pg exposure alters the balance of proximal versus distal nephron cell fates, such that the PCT is abrogated, while the PST is promoted at the expense of the DL. Together, these data suggest that both RA and Pg have effects on kidney patterning that may have translational merit for diseases like CAKUT.

68. Chemical Genetic Screening in Zebrafish Identifies Nitric Oxide Signal and Histone Acetylation to Coordinate Neural Crest Development and Craniofacial Morphogenesis. ***Yawei Kong***[1,2,3,5], *Michael Grimaldi*[1,2,3,5], *Eugene Curtin*[1,2], *Max Dougherty*[1,2], *Charles Kaufman*[4], *Richard White*[4], *Leonard Zon*[4,5], *Eric Liao*[1,2,3,5]. 1) Center for Regenerative Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA; 2) Division of Plastic and Reconstructive Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA; 3) Shriners Hospitals for Children, Boston, MA 02114, USA; 4) Howard Hughes Medical Institute, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA; 5) Harvard Stem Cell Institute, Boston, MA 02114, USA.
Cranial neural crest (CNC) cells are patterned and coalesce to facial prominences that undergo convergence and extension to generate the craniofacial form. We applied a chemical genetics approach to identify pathways that regulate craniofacial development during embryogenesis. Treatment with the nitric oxide synthase inhibitor TRIM abrogated first pharyngeal arch structures and induced ectopic ceratobranchial formation. TRIM promoted a progenitor CNC fate and inhibited chondrogenic differentiation, which were mediated through impaired nitric oxide (NO) production without appreciable effect on global protein S-nitrosylation. Instead, TRIM perturbed hox gene patterning and caused histone hypoacetylation. Rescue of TRIM phenotype was achieved with over-expression of histone acetyltransferase kat6a, inhibition of histone deacetylase, and complimentary NO. These studies demonstrate that NO signaling and histone acetylation are coordinated mechanisms that regulate CNC patterning, differentiation and convergence during craniofacial morphogenesis.

69. Behavior-based screening identifies compounds controlling the switch between passive and active threat responses. ***A.J. Rennekamp***[1,2], *S. Patel*[1,2], *P.J. Lorello*[3], *X.P. Huang*[4], *L. Cade*[1,2], *B.L. Roth*[4], *B.J. Caldarone*[3], *D. Kokel*[1], *R.T. Peterson*[1,2]. 1) Cardiovascular Research Center and Division of Cardiology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA; 2) Broad Institute, Cambridge, MA; 3) Department of Neurology, Brigham and Women's Hospital, NeuroBehavior Laboratory, Harvard NeuroDiscovery Center, Boston, MA; 4) National Institute of Mental Health Psychoactive Drug Screening Program, University of North Carolina Chapel Hill Medical School, Chapel Hill, NC.
Freezing in response to aversive stimuli is a behavioral trait observed in most animals. In zebrafish larvae, freezing can be induced robustly by exposure to strobe light. To examine the biological basis for innate freezing, we developed a high-throughput behavioral assay and systematically characterized the effects of more than 3,500 small molecules with known pharmacology. We found that dopaminergic, cholinergic and GABAergic drugs are major modulators of animal freezing. Several of these compounds completely switch the behavioral phenotype from freezing to escape-like behavior. Freezing is restored by addition of antipsychotics, anxiolytics and anticholinergics. We further exploited the assay to screen 10,000 synthetic compounds and identified several new neuroactive compounds including a structurally novel class of small molecules with neurological activity in mice. Thus, systematic pharmacological interrogation of the freezing response reveals critical underlying mechanisms and offers an efficient path to discovery of novel neuroactive compounds.

70. Systematic phenotype prediction to accelerate reverse genetic and small-molecule screening. ***Gabriel Musso***[1,2], *Murat Tasan*[3,4], *Frederick Roth*[3,4], *Calum MacRae*[1,2]. 1) Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Lunenfeld-Tanenbaum Research Institute, Toronto, ON; 4) University of Toronto, Toronto, ON.
Determination of gene or small molecule function through analysis of associated phenotypes is a mainstay of modern molecular biology. However, given the number of observable phenotypes in embryonic zebrafish, a comprehensive analysis of perturbation effects is untenable. The purpose of our work has been to develop unbiased phenotype prediction methods, the results of which would highlight potential gene or small molecule phenotypes before screening, and could increase the pace and sensitivity of experimentation. We began by prioritizing genes for morpholino-based phenotypic assay in zebrafish through genomic data integration and machine learning, predicting the effect of loss of function of each of 15,106 zebrafish genes on 338 distinct embryonic anatomical processes. The results of these predictions have been made freely available through a searchable web browser (http://zfunc.mshri.on.ca) and through the functional analysis tool GeneMANIA (www.genemania.org). Cross-validation suggested the resulting predictions to be particularly precise in identifying both morphant and mutant phenotypes, and notably, at predicting disease association for corresponding human orthologs. In proof-of-concept studies we validated 16 high-confidence cardiac predictions using targeted morpholino knockdown. Among these was tmem88a, a recently described attenuator of Wnt signaling, which we found to be a discrete regulator of the patterning of intercellular coupling in the zebrafish cardiac epithelium. Similarly, by systematically annotating observable phenotypes following treatment with each of over 4,000 bioactive small molecules, we found that compound-phenotype association can be predicted in a learning framework based on chemical properties, enabling accurate in silico screening of hundreds of thousands of compounds for specific zebrafish phenotypes. Thus, systematic phenotype prioritization in zebrafish can accelerate the pace of developmental gene function and de novo lead compound discovery.

71. Adaptive changes in vibration attraction behavior and its sensory receptors promote eye degeneration in the cavefish, *Astyanax mexicanus*. ***Masato Yoshizawa***[1], *Kelly O'Quin*[2], *Alex Keene*[1], *William Jeffery*[3]. 1) Biology, Univ. Nevada, Reno, Reno, NV; 2) Biology, St. Bonaventure Univ., St. Bonaventure, NY; 3) Biology, Univ. Maryland, College Park, MD.
Cave-dwelling fish show remarkable changes in many traits including foraging and sensory-based behaviors to adapt to the dark cave environment. *Astyanax mexicanus* is a model cave-dwelling fish with sighted surface-dwelling (surface fish) and blind cave-dwelling (cavefish) forms. The surface ancestors of cavefish were trapped in caves million years ago and altered multiple morphological and behavioral traits. We have identified a dramatic increase in vibration attraction behavior (VAB), the ability of fish to swim toward the source of a water disturbance in darkness in independently derived cavefish populations. We found that VAB is controlled by several genetic factors, has an advantage for feeding in the dark, and has its tuning peak at 35 Hz, which is produced by prey. Therefore, VAB is an adaptive foraging trait that evolved in the dark, food-sparse cave environment. We also found that this foraging behavior is mediated by superficial neuromasts, which are increased in size and number in cavefish. F_2 hybrids derived from a surface fish × cavefish cross indicate

that superficial neuromasts restricted to the cavefish eye orbit are genetically correlated with eye reduction and VAB. Ablation experiments demonstrate a major role for these sensory receptors in VAB expression. Genetic analysis using genotyping-by-sequencing technology revealed that quantitative trait loci (QTL) for VAB, neuromast at the eye orbit, and reduced eye size form two congruent clusters on *Astyanax* genome. Finally, we also show that, like *Astyanax* surface fish, zebrafish lack VAB, suggesting that this trait may be unique to cave-dwelling organisms. We conclude that natural selection for VAB and superficial neuromast enhancement may be an indirect cause of eye regression in cavefish through genetic linkage or antagonistic pleiotropy. These results show how interdisciplinary approaches can lead to new insights into the evolution of adaptive behaviors.

72. Pigment cell heterochronies underlying pattern evolution in *Danio* fishes. ***Larissa Patterson***, *Emily Bain*, *David Parichy*. University of Wasington, Seattle, WA.
Teleost fishes have diverse and striking pigment patterns, yet we know little about how evolutionary changes in pigment cell development generate pattern variation. As an adult the zebrafish, *Danio rerio* has a pattern of dark horizontal stripes of melanophores alternating with light interstripes of yellow xanthophores and iridescent iridophores. In contrast, *D. albolineatus* has an evolutionarily derived, uniform pattern in which melanophores, xanthophores and iridophores are intermingled. Here we show that iridophores not only initiate and orient stripes in *D. rerio*, but also form boundaries that terminate developing stripes. In *D. albolineatus*, however, very few iridophores develop whereas xanthophores develop precociously and over a much wider area than in *D. rerio*. This difference in xanthophore development is associated with similarly early and widespread expression of the xanthogenic factor Colony stimulating factor-1 (Csf1), owing to *cis*-regulatory evolution at the Csf1a locus. Finally, we show that expressing Csf1 similarly to *D. albolineatus* in *D. rerio* results in melanophore and xanthophore intermingling, loss of interstripe iridophores and a pattern resembling that of *D. albolineatus*. Together our analyses indicate that evolutionary changes in Csf1 expression and concomitant alterations in the development of all three pigment cell classes have likely contributed to the loss of stripes in *D. albolineatus*. These results further suggest that changes in the timing of pigment cell differentiation can have cascading effects on pattern development and may have contributed to evolutionary diversification in this group.

73. Phylo-Mapping: a comparative genomic approach to understand natural variation. ***Jacob Daane***[1,2], *Matthew Harris*[1,2]. 1) Harvard Medical School, Boston, MA; 2) Boston Children's Hospital, Boston, MA.
The diversity in Nature is akin to a genetic screen through which many interesting, biomedically and industrially relevant genetic perturbations may be revealed. However, comparative genomic approaches toward isolating these genetic changes are predominantly limited to species for which we have existing genetic resources. To facilitate analysis of diverse and rare species, we are developing a pipeline for whole exome analysis from fresh or archived DNA samples. Using the reference sequence of a related species as bait, we perform targeted capture to enrich DNA libraries for conserved, orthologous protein-coding genes in another species. Sequencing reads are then assembled into contigs, and the ancestral reference sequence is reconstructed for a clade as a scaffold for comparative analysis. To test this approach, we sequenced members of a monophyletic group, *Phoxinellus spp.*, where loss of scales is a defining and derived trait. We then identified lineage-specific patterns of positive selection and/or drift within *Phoxinellus* relative to a closely related, scaled sister taxa, *Telestes*. Our analysis identified fixation of predicted deleterious polymorphisms in *Phoxinellus fgfr1a*. Interestingly, alteration in *fgfr1a* function is sufficient for scale-loss in zebrafish. We further identified fixed non-synonymous variants in *fgf20a*, a ligand of *fgfr1a*, suggesting loss of this signaling 'cassette' in the evolution of scale loss in this group. Mutations in *fgf20a* (*dob*) in zebrafish have no clear effect on scale patterning. However, when the *fgf20a*^{dob} mutant is crossed to a sensitized zebrafish background carrying loss-of-function *fgfr1a* mutations (*spd*), the combined reduction in ligand and receptor is sufficient to reduce the size and number of scales. Thus, despite no prior genetic information from these species, we have identified fixed changes associated with the evolution of scale-reduction in the *Phoxinellus* genus and demonstrated a genetic interaction between *fgf20a* and *fgfr1a* in scale development. This "phylo-mapping" approach enables testable predictions of the signaling processes and potential ecological pressures associated with character change in an evolving trait.

74. Resolution of the *Danio* Phylogeny through RAD Phylogenomics. ***Braedan M. McCluskey***, *Peter Batzel*, *John H. Postlethwait.* Institute of Neuroscience, University of Oregon, Eugene, OR.
Recent phylogenetic studies of the *Danio* genus have inferred conflicting phylogenies with low support for several important relationships including the closest extant relative(s) of *Danio rerio*. To bring consensus to the relationships within the Danio genus, we performed phylogenomic analyses using Restriction site Associated DNA Sequencing (RAD-Seq). Our analyses, which utilize sequence information from more than 30,000 orthologous RAD-tag loci mapped to the zebrafish genome, provide sufficient phylogenetic signal to unambiguously resolve the *Danio* phylogeny and provide strong evidence for *Danio aesculapii* as the closest extant relative of zebrafish. These results are consistent across analytical methods and multiple datasets (both genome-assisted and genome-independent). Across RAD-tag loci, however, the data are inconsistent with a single underlying topology and suggest the diversification of the *Danio* genus involved speciation with gene flow and/or considerable levels of introgression. In addition to inferring a well-supported topology for *Danio*, we performed a characterization of the mapping locations and degree of conservation of the RAD-tag loci in the zebrafish genome. We demonstrate that these loci can be retained across several million years of evolution, are concentrated near exons, and display varying levels of conservation in specific regions of the genome. These data also suggest that the highly repetitive nature of the zebrafish genome is partially due to a recent expansion of some families of repetitive elements during the diversification of the *Danio* genus. To verify the results of this RAD-Seq approach and begin comparative protein analyses across the genus, we also analyze sequences from protein-coding regions of zebrafish and some of its closest relatives. Together, these results provide a high-resolution profile of the genome-wide diversification of the *Danio* genus and provide further tools for the understanding of zebrafish in an evolutionary context.

75. The evolution of morphological novelty through the EDA-mediated regionalization of skin appendages. ***M. Brent Hawkins***[1,2], *Alexandra Rodriquez*[2], *Katrin Henke*[1], *Sven Reischauer*[3], *Matthew P. Harris*[1], *David W. Stock*[2]. 1) Boston Children's Hospital, Harvard Medical School, Boston, MA; 2) Department of Ecology & Evolutionary Biology, University of Colorado, Boulder, CO; 3) Developmental Genetics, Max Planck Institute for Heart & Lung Research, Bad Nauheim, Germany.
The patterning of skin appendages into organized arrays, such as feathers into tracts and teeth into dentitions, is critical for their function, but the genetic mechanisms that underlie the development of such regional organized arrays within an epidermis are incompletely understood. To elucidate these mechanisms, we have investigated zebrafish breeding tubercles, multicellular keratinized skin appendages with roles in mating, that exist as both individual organs as well as contiguous arrayed tubercle tracts. Phylogenetic analyses reveal that individual tubercles are present throughout Cypriniformes (the order containing zebrafish), but specific tracts of arrayed tubercles arose as morphological novelties within the genus *Danio*. We examined tract development in wild type and mutant backgrounds, and found that the ectodysplasin (EDA) signaling pathway is key to tract formation and patterning. EDA pathway mutants developed smaller tracts with fewer tubercles compared to wild type siblings, while the morphology of tubercles themselves was unaffected. Mutants also showed decreased activity of NFkB, an EDA signaling effector, at the tract margin. These results suggest that EDA signaling is necessary for tract patterning but not tubercle morphogenesis. Next, we looked at the effect of ectopic EDA ligand overexpression using the Tg[ef1a:EDA] line. Transgenic animals developed larger tracts with more tubercles and expanded NFkB activation compared to siblings. Additionally, transgenic animals developed ectopic tracts on the head, fins, and scales, indicating that EDA activity is sufficient to specify tract formation in naïve ectoderm. The ability of *eda* to form new tracts in previously non-tract skin suggests this pathway may have played a role in the origin of novel, localized, arrayed tubercle tracts in *Danio*. Furthermore, our results suggest candidate mechanisms that may act in the regional organization and patterning of skin appendages in other vertebrate lineages.

76. Transcriptional control mediated by cohesin and Ctcf in early zebrafish development. *Michael Meier*[1], *Jenny Rhodes*[1], *Justin M. O'Sullivan*[2], ***Julia A. Horsfield***[1]. 1) Department of Pathology, DSM, University of Otago, Dunedin, New Zealand; 2) Liggins Institute, The University of Auckland, Private Bag 90219, Auckland, New Zealand.
The genome and the epigenome act in concert to regulate the formation of a diverse array of cell types during development, but how this happens is not clear. Cohesin and CCCTC-binding factor (Ctcf) are architectural proteins with chromatin organizing functions, and have important roles in the regulation of gene transcription. We hypothesize that cohesin and Ctcf have distinct roles at the onset of zygotic gene expression to organize chromatin architecture, and that this activity helps determine cell fate. We used ChIP-seq in the vertebrate model *Danio rerio* (zebrafish) to map genome-wide binding of Ctcf and cohesin at the onset of global zygotic genome activation. In addition, we used RNA-seq to analyze the transcriptional consequences of knocking down these proteins in early developmental stages. We detected cohesin binding at genes encoding core transcription factors Oct4, Nanog, and Sox, polycomb group (PcG) proteins (*bmi1*, *pcgf5a*, and *epc2*), histone-modifying enzymes (*setd1ba* and *suds3*), and histone variants (*h3f3a*). Furthermore, Gene Ontology analysis revealed that the biological process categories of nucleosome assembly, chromatin assembly, nucleosome organization, protein-DNA complex assembly, and protein-DNA complex subunit organization were the top eight categories significantly associated with cohesin binding. Differential gene expression analysis of the RNA-seq datasets showed that cohesin and Ctcf depletion had positive as well as negative effects on transcription. Furthermore we detected cohesin- and Ctcf- dependent alternative splicing events raising the possibility that these proteins contribute to transcript diversity in the early embryo.

77. Cardiomyocyte-specific profiling of gene-regulatory programs during heart regeneration using the replacement histone H3.3. ***Joseph A. Goldman***[1], *Amy L. Dickson*[1], *Jaclyn M. Karasik*[1], *Sean Thomas*[2,3], *Benoit G. Bruneau*[2,3], *Kenneth D. Poss*[1]. 1) Cell Biology, Duke Medical Center, Durham, NC; 2) Gladstone Institute of Cardiovascular Disease; 3) University of California, San Francisco.
Chromatin organization is a principal mechanism to institute changes in cell differentiation. Recently, genome-wide methods for analyzing chromatin features have become available for observing gene regulatory events at high resolution. Adapting such technologies to complex tissues *in vivo* is limited because of the difficulty in isolating the cell type of interest away from other cells that might confound the analysis. Here we have taken advantage of the properties of histone H3.3, deposited within active gene bodies and transcriptional control loci, to identify dynamic gene regulatory events during zebrafish heart regeneration. First, we generated transgenic zebrafish in which H3.3 is expressed and biotinylated specifically in cardiomyocytes. Then, we established methodology to profile H3.3 occupancy in cardiomyocytes within embryonic hearts, adult hearts, and hearts undergoing injury-induced regeneration. With this technology, we generated tissue-specific, genome-wide profiles of H3.3 occupancy in different key contexts of cardiomyocyte biology, and we identified many genes and distal regulatory elements that are differentially regulated in these contexts. Our experiments demonstrate the power of H3.3 profiling to define the activity of gene regulatory elements in specific cell types within complex tissues, an approach that is adaptable to diverse biological questions.

78. The capture and analysis of cardiac lineage-specific and stage-specific gene expression profiles in zebrafish. ***Todd A. Townsend***, *Bushra Gorsi*, *H. Joseph Yost.* Molecular Medicine Program, University of Utah, Salt Lake City, UT, USA.
While several critical components of cardiovascular development have been elucidated, the gene expression profile and signaling mechanisms governing these processes is largely undetermined. Temporal and lineage-specific regulation of gene expression occurs throughout development and in response to distinct specific cell signaling events; however, current technology is constrained by the inability to effectively collect purified lineages at early developmental timepoints. To circumvent this problem we developed both transgenic zebrafish lines with biotin ligase recognition peptide (BLRP) fused to a ribosomal protein (Rpl), and lines in which lineage-specific expression of biotin ligase (BirA) in vitro biotinylates BLRP-Rpl. This BLRP-Rpl-BirA technology allows us to capture polysomes from specific cell lineages out of whole embryo lysates with high affinity. Isolated lineage-specific polysomes provide mRNAs for RNA-seq analysis, to determine gene expression profiles from distinct tissues/cell lineages at specific developmental timepoints. Using a cardiac

lineage specific (cmlc2/myl7) promoter to drive BirA expression, we have captured BLRP-biotin-tagged polysomes from developing cardiac lineages as early as bilateral heart primordia stage, and performed RNA-Seq and RT-PCR analysis. In addition, we are generating lines that drive expression of BirA and BLRP-Rpl biotinylation in specific cardiac sublineages and in response to distinct cell signaling pathways. This powerful technique has allowed us to obtain differential genome-wide expression profiles within cardiac lineages at specific timepoints that are critical for embryonic heart development. Supported in part by AHA 09POST2260423 and NHLBI 5F32HL114181 postdoctoral fellowships, and U01HL098179 (NHLBI Bench-to-Bassinet program).

79. Analyzing multigenerational effects of the Piwi/piRNA pathway in zebrafish. ***Holger Dill***, *Rene' Ketting*. Institute of Molecular Biology, Mainz, Germany.
Novel integration of mobile DNA elements contributes substantially to eukaryotic genome evolution. Nevertheless, transposon insertions into protein coding gene loci can in many cases be harmful for host organisms. Germ cells are responsible for transmission of intact genetic information to subsequent generations. To counteract the proliferation of transposons and to protect germ cell genomes from damage, the animal germ line has developed an RNAi based immune system to silence these elements, the Piwi-interacting RNA (piRNA) pathway. In contrast to other small regulatory RNAs only little is known about (1) piRNA biogenesis, (2) the piRNA target repertoire and (3) cell type specific functions of piRNAs. To analyze the basic functions of vertebrate piRNAs under highly controlled conditions we generated zebrafish lines expressing piRNAs with a unique sequence targeting a single copy transgene, the germ cell specific *vasa*::EGFP. To this end, we randomly introduced EGFP-modified Tol2 DNA-transposons into the zebrafish genome to trigger a piRNA response against EGFP. Integration of these EGFP-containing transposons leads to biogenesis of small RNAs mapping to the Tol2-EGFP transgene. Small RNA sequencing revealed that these GFP-piRNAs resemble characteristics of endogenous primary and secondary piRNAs very well. In these fish lines a *trans*-silencing of the *vasa*::EGFP target gene could be observed which is stable over at least four generations. Interestingly, a strong piRNA response could be observed within one generation after infiltration of the fish population by the Tol2-EGFP. The observed silencing is inherited to offspring in a strictly maternal fashion, presumably by maternal piRNAs. EGFP expression in somatic tissues, e.g. cardiomyocytes or spinal motor neurons is not affected indicating a germ cell specific function of piRNAs in zebrafish. By using this *in vivo* system, we are currently investigating piRNA synthesis from distinct genomic loci (so-called piRNA clusters), how the piRNA response to recently introduced transposons is initiated and by which mechanism piRNAs actually silence their target genes in vertebrate species (transcriptional/posttranscriptional gene silencing, chromatin remodeling etc.).

80. Single cell transcript counting. ***Steven Harvey***, *John Collins, Neha Wali, Ian Sealy, Jorge Zamora, Ian Packham, Derek Stemple.* The Wellcome Trust Sanger Institute, Cambridge, UK.
In recent years RNA-seq has revolutionised our ability to study RNA biology. However, such methods have been dependent on large quantities of starting RNA and therefore usually performed on pools of many cells. Subsequently, while RNA-seq has successfully been used to determine that a given gene is expressed at a specific developmental time or within a tissue, the heterogeneity of the cells within those tissues cannot be deciphered. Understanding the expression levels of genes from one cell to another is critical, as these changes will have important functional consequences. Advancements in using small quantities of starting RNA have made single cell transcriptome studies possible. I will present a novel single cell RNA sequencing method, based on the 3' enrichment of mRNAs. Using unique molecular identifiers (UMI) each mRNA molecule is given a signature that allows unique mRNAs to be distinguished from PCR amplified molecules. The method provides a robust and more absolute quantitative measurement of mRNA levels within single cells. By adding equal quantities of a range of exogenous RNAs to each single cell library preparation we are able to distinguish technical variation from biological variation and therefore identify genes that have highly variable expression levels. We are now generating 96 well plates of single cell libraries to address specific biological questions ranging from investigations into the dynamics of single cell transcript profiles to the detection of unique cell populations.

81. Serotonin Regulates Midline Axon Crossing via EphrinB2. ***Lingyan Xing***[1,2,3], *Jong-hyun Son*[1,2], *Tamara Stevenson*[1,2], *Tiffanie Dahl*[1,2,3], *Josh Bonkowsky*[1,2,3]. 1) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, United States of America; 2) Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah, United States of America; 3) Interdepartmental Program in Neurosciences, University of Utah School of Medicine, Salt Lake City, Utah, United States of America.
Serotonin neurons and their axon projections are widespread in the central nervous system (CNS) in early development. Disruption of serotonergic signaling leads to diffuse CNS abnormalities with a wide range of behavioral phenotypes. This suggests a non-classical role for serotonin in development. In mice, serotonin can modulate axon responsiveness to netrin-1, causing thalamocortical axons to adopt a more ventral pathway and altered fasciculation of medial pre-frontal cortex neurites. While serotonin appears necessary for axon guidance, no one has explored its role in commissure formation. Moreover, the mechanism of serotonin action in pathfinding is poorly understood. To determine the role of serotonin in commissure formation and characterize the molecular mechanism, we used embryonic zebrafish to visualize axon guidance decisions, in transgenic lines with precise expression in subsets of well-defined axon pathways. Using pharmacological blockade of serotonin signaling, genetic ablation of the serotonergic neurons, or knock-down of serotonin receptors, we found that axons of telencephalic neurons fail to cross the midline, indicating a role for serotonin in commissural axon guidance. We further demonstrate that serotonin acts through its main excitatory G protein-coupled receptor htr2a. Blockade of htr2a elevates axonal receptor-tyrosine kinase ephrinB2 levels, causing failure of commissural axon midline crossing. Thus, our study demonstrates an unexpected instructive role for serotonin in axon pathfinding choice, and identifies ephrinB2 as a key mediator in serotonin-regulated pathfinding.

82. The adhesion GPCR Gpr126 has distinct, domain-dependent functions in Schwann cells governed by interaction with Laminin-211.
Sarah C. Petersen[1], *Rong Luo*[2,3], *Amit Mogha*[1], *Torsten Schöneberg*[4], *Ines Liebscher*[4], *Xianhua Piao*[2,3], *Kelly R. Monk*[1]. 1) Washington University in St. Louis School of Medicine; 2) Boston Children's Hospital; 3) Harvard Medical School; 4) University of Leipzig.
Myelin ensheathes vertebrate axons to allow rapid propagation of action potentials and provide trophic support of neurons. In the peripheral nervous system (PNS), myelin is formed by Schwann cells (SCs), which radially sort axons into a 1:1 relationship before wrapping an axonal segment. Myelination in the PNS of both zebrafish and mice requires Gpr126, an orphaned adhesion family G protein-coupled receptor (aGPCR). As an aGPCR, Gpr126 is purported to have dual roles in cell-cell and/or cell-matrix interactions via its large extracellular N-terminal fragment (NTF), and in signal transduction with its 7-pass transmembrane C-terminal fragment (CTF). We are using TALENs to generate an allelic series of zebrafish mutants for structure-function analysis of Gpr126. Our mutant analyses support a model in which the Gpr126 NTF is necessary and sufficient for radial sorting, while the CTF alone elevates cAMP to promote myelination. Furthermore, our biochemical analyses are consistent with the hypothesis that the Gpr126 NTF binds SC-derived Laminin-211 to promote myelination. We have validated this model *in vivo* with *lama2* loss- and gain-of-function experiments in zebrafish to show that *lama2* genetically interacts with the *gpr126* myelination program. Finally, G protein signaling assays in heterologous cells demonstrate that Laminin-211 functions as a molecular switch between Gpr126-mediated Gi and Gs signaling, which we predict are required for radial sorting and myelination, respectively. We are also performing unbiased genetic and chemical enhancer/suppressor screens in zebrafish to identify additional interactors with *gpr126*. Our screens have revealed multiple candidate mutants and small molecules that enhance or suppress a hypomorphic allele of *gpr126*. Thus, our work lends insight into the mechanistic basis of Gpr126 function in myelination and identifies interacting genes and small molecules that play a crucial role in Schwann cell development and myelination.

83. Calsyntenin-1 Regulates Axon Branching and Endosomal Trafficking During Sensory Neuron Development *in vivo*. ***O.Y. Ponomareva***, *I.C. Holmen, A.J. Sperry, K.W. Eliceiri, M. C. Halloran.* University of Wisconsin, Madison, Madison, WI.
Precise regulation of axon branching is crucial for neuronal circuit formation, yet the mechanisms that control branch formation are not well understood. Moreover, the highly complex morphology of neurons makes them critically dependent on protein/membrane trafficking and transport systems, although the functions for membrane trafficking in neuronal morphogenesis are largely undefined. We are investigating mechanisms regulating axon branching and differential guidance of separate axons from one neuron, using zebrafish sensory neurons as a model. We identify a kinesin adaptor, Calsyntenin-1 (Clstn1), as an essential regulator of axon branching and neuronal compartmentalization *in vivo*. We use morpholino knockdown and a TALEN-generated Clstn1 mutant to show that Clstn1 is required for formation of peripheral, but not central, sensory axons, and for peripheral axon branching. We also use live imaging of endosomal trafficking *in vivo* to show that Clstn1 regulates transport of Rab5 containing endosomes from the cell body to specific locations of developing axons, and that differential endosome dynamics may help to define sensory neuron compartmentalization. Our results suggest a model in which Clstn1 patterns separate axonal compartments, and defines their ability to branch by directing trafficking of specific endosomes.

84. Anterograde axonal transport involvement in synaptic function and growth coordination. ***Thomas O. Auer***[1,2], *Tong Xiao*[3], *Valerie Bercier*[1], *Christoph Gebhardt*[1], *Karine Duroure*[1], *Jochen Wittbrodt*[2], *Herwig Baier*[4], *Filippo Del Bene*[1]. 1) U934/UMR3215, Institute Curie, Paris, France; 2) Centre for Organismal Studies, University of Heidelberg, Heidelberg, Germany; 3) University of California, San Francisco, USA; 4) Max Planck Institute of Neurobiology, Martinsried, Germany.
Axonal transport is crucial for the correct development of neuronal processes and neuronal function but its exact role in vivo is still poorly understood. Utilizing TALE nucleases we generated a loss-of-function allele of a zebrafish orthologue of the anterograde motor kinesin 5. Homozygote mutants show expanded melanocytes and are completely blind. As kinesin 5 is expressed in retinal ganglion cells and retinal patterning is not affected, we found a compromised development of proper synaptic connections between the eye and the brain targeting areas including the optic tectum. Mutant analysis revealed a specific delay in retinotectal projections development with immature processes present until day 5. Anterograde transport of organelles and vesicles is affected in mutant axons as revealed by in vivo imaging. Surprisingly, single cell analysis showed that later retinal ganglion cells develop dramatically extended axonal branches due to failure of normal pruning and excessive axonal growth. We could confirm that this excessive growth is mediated by a lack of functional innervation of target areas that consequently produce an excess of neurotrophic factors. Using the kinesin 5 mutation as tool, we propose a novel role of neurotrophic factors in activity dependent tissue growth coordination between the retina and the optic tectum.

85. Regulation of dynein-mediated mitochondrial transport in axons. ***Catherine Drerup***, *Alex Nechiporuk.* Department of Cell and Developmental Biology, Oregon Health & Science University, Portland, OR.
The formation and maintenance of neural circuits requires the active transport of proteins and organelles in neural processes. While anterograde movement (*away* from the cell body) utilizes the kinesin superfamily of motor proteins, retrograde transport (*towards* the cell body) is accomplished primarily by one motor, cytoplasmic dynein. The diversity of cargos moved by dynein and the regulation of their deposition implies the existence of cargo-specific modulators of retrograde movement. One cargo whose retrograde movement is essential for axon formation and maintenance is mitochondria. These organelles are transported to sites with high metabolic demands and synaptic activity as they supply energy and maintain calcium homeostasis. A large body of work has elucidated the molecular machinery necessary for anterograde movement of mitochondria; however, how these organelles attach to the dynein complex for retrograde transport remained unknown. In a genetic screen, we isolated a zebrafish mutant with mitochondrial accumulations in axon terminals due to defects in retrograde mitochondrial movement. We have determined that this defect is specific as: 1) dynein localization and movement are normal; 2) the localization of other cargo is unaffected; and 3) mitochondrial anterograde transport frequency is normal. Using RNAseq-based cloning techniques, we identified the causative genetic lesion as a T to G mutation in the start codon of the gene encoding Actr10, a member of the dynein-interacting complex dynactin. Though this protein is ideally situated to serve as a cargo adaptor, a role for Actr10 in

cargo transport has not been reported. Our data indicate that Actr10 has a critical role in attaching mitochondria to the retrograde motor complex, which, when disrupted, leads to mitochondrial accumulation in dysmorphic axon terminals. Using a combination of biochemistry and *in vivo* imaging, future work will identify the mitochondrial-associated protein which links this organelle to the Actr10-dynein complex. The culmination of this work will determine how Actr10-mediated transport of mitochondria regulates the proper positioning of this organelle, allowing the maintenance of functional neural circuits.

86. The Role of Planar Cell Polarity in Directed Cell Migration. ***Andrew W Mathewson***[1,2], *Crystal Davey*[1,2], *Cecilia Moens*[1,2]. 1) University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.
During development, neurons must migrate from their birthplace to their functional destinations in the brain in order to create working neural networks. In zebrafish and other vertebrates, the facial branchiomotor neurons (FBMNs) undergo a well-characterized migration in the plane of the hindbrain neuroepithelium from rhombomere 4 to rhombomere 6. Rather than identifying chemotropic signals or receptors, forward genetic screens in the zebrafish for FBMN migration mutants have identified mutations in almost all of the core components of the Planar Cell Polarity (PCP) pathway (Vangl2, Fz3a, Pk1b, Celsr3 and Scrib). PCP signaling has also been implicated in neuron migration in other contexts in vertebrates and invertebrates. The PCP pathway is a cell-contact dependent mechanism for generating polarity in the plane of an epithelium, but how PCP controls highly dynamic processes like neuronal migration is not understood. FBMNs contact a variety of tissues during their migration including the segmented hindbrain neuroepithelium, the floor plate, as well as other migrating FBMNs. Where PCP signaling is required for FBMN migration is controversial. Most studies have identified a cell-non-autonomous role for PCP, but which cells promote FBMN migration, and how they use the PCP pathway to do so, is unknown. Furthermore a cell-autonomous role for PCP signaling within the FBMNs has been demonstrated in some studies but refuted in others. Using the Gal4/UAS system and PCP-specific dominant-negative forms of Dishevelled (DN-Dvl) and Fz3a (DN-Fz3a) to systematically disrupt PCP in a cell-type and rhombomere-specific manner, we present evidence of both a cell-autonomous requirement for PCP within FBMNs and a non-cell autonomous requirement specifically in the rhombomere 4 environment in which the FBMNs arise. Using high-resolution single-cell timelapse imaging in vivo, we localize PCP proteins to FBMN filopodial tips and demonstrate a novel role for PCP signaling in FBMN filopodial dynamics. Together, our findings suggest a model in which PCP signaling between the planar polarized neuroepithelial environment and the nascent FBMNs initiates their directional migration by the selective stabilization of FBMN filopodia.

87. Intraciliary calcium waves initiate vertebrate left-right asymmetry. *Shiaulou Yuan*[2], *Lu Zhao*[1], *Martina Brueckner*[2], ***Zhaoxia Sun***[1]. 1) Genetics, Yale University School of Medicine, New Haven, CT; 2) Pediatrics, Yale University School of Medicine, New Haven, CT.
Directional cilia-driven fluid flow at the left-right organizer (LRO) breaks bilateral symmetry during vertebrate development. Yet how flow is sensed and directs downstream events remains unclear. We now identify the primary cilium as a functional calcium signaling compartment and demonstrate that polycystin2 (Pkd2), a cilia-localized cation channel, initiates intraciliary calcium waves at the LRO to direct left-right patterning. Utilizing cilia-targeted genetically-encoded calcium indicators, we discover intraciliary calcium waves in-vivo at the zebrafish LRO that are dynamic, asymmetric and Pkd2-dependent. Further, we show that asymmetric intraciliary calcium is upstream of asymmetric mesendodermal calcium in left-right development and coincides with the onset of ciliary motility at the LRO, making it the earliest asymmetric molecular signal. Finally, we suppress intraciliary calcium and demonstrate that it is essential for left-right development. These findings demonstrate that intraciliary calcium determines left-right development and identify cilia as a specialized ion signaling compartment critical for body axis patterning.

88. Prostaglandin signaling regulates ciliogenesis by modulating intraflagellar transport. *Daqing Jin*[1], *Terri T. Ni*[2,3], *Jianjian Sun*[1], *Guangju Yu*[1], *Haiyan Wan*[2], *Jeffrey D Amack*[4], *Jonathan Fleming*[3], *Chin Chiang*[3], *Satish Cheepala*[5], *Gwenaëlle Conseil*[6], *Susan P.C. Cole*[6], *Iain Drummond*[7], *John D. Schuetz*[5], *Jarema Malicki*[8], ***Tao P. Zhong***[1,2]. 1) State Key Laboratory of Genetic Engineering, Department of Genetics, Fudan University School of Life Sciences, Shanghai, China; 2) Department of Medicine, Vanderbilt University School of Medicine, USA; 3) Department of Cell & Developmental Biology, Vanderbilt University School of Medicine, USA; 4) Department of Cell & Developmental Biology, State University of New York Upstate Medical University,USA; 5) Department of Pharmaceutical Science, St. Jude Children's Research Hospital, USA; 6) Division of Cancer Biology and Genetics, Queen's University, Kingston, ON K7L3N6, Canada; 7) Department of Medicine, Massachusetts General Hospital, Harvard Medical School, USA; 8) MRC Center for Developmental and Biomedical Genetics, The University of Sheffield, United Kingdom.
Cilia are microtubule-based organelles that mediate signal transduction in various tissues. They are assembled and extended by bidirectional movement of intraflagellar transport (IFT). Here we report that prostaglandin signaling is crucial to ciliogenesis by regulating anterograde IFT. We analyzed the zebrafish leakytail (lkt) mutant that displays ciliogenesis defects and cilia-associated malformations. Positional Cloning reveals that lkt encodes an ATP-binding cassette transporter ABCC. We found that ABCC localizes on the cell membrane and exports prostaglandin E2 (PGE2). Consistent with the above findings, PGE2 synthesis enzymes, COX-1 and COX-2, and its receptor, EP in the cilium, are required for proper cilia formation and elongation. Conservatively, depletion of ABCC or EP in human retinal pigment epithelial cells suppresses ciliogenesis and recapitulates lkt ciliary phenotypes. Live imaging of IFT particles in cells reveals that PGE2 signaling increases anterograde but not retrograde velocity of IFT. These findings lead us to propose that ABCC-mediated PGE2 signaling acts through a ciliary G-protein-coupled receptor, EP, to upregulate cAMP synthesis and increase anterograde IFT, thereby promoting ciliogenesis.

89. BBS chaperonin complex function in zebrafish transport and vision. ***Charles Scott***[1], *Denise Oh*[1], *Daniel Grigsby*[1], *Trudi Westfall*[1], *Xitiz Chamling*[2,3], *Diane Slusarski*[1]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Interdisciplinary Graduate Program in Genetics; 3) Department of Pediatrics, University of Iowa, Iowa City, IA.
Bardet-Biedl syndrome (BBS) is a human genetic disorder characterized by symptoms including, but not limited to, retinal degeneration, obesity, polydactyly, and polycystic kidneys. The range of symptoms is a result of defects in the maintenance and function of the cilia. The primary cilium is a necessary structure for proper cellular signaling during development as well as during adulthood. To date 18 BBS genes have been identified and form two main protein complexes that are necessary for proper cilia maintenance. BBS 1, 2, 4, 5, and 7-9 form a stable complex known as the BBSome which is involved in protein trafficking up within the cilia. BBS 6, 10, and 12 form a complex with other chaperones which is necessary for assembly of the BBSome. Our lab has developed the zebrafish as a model system to study the function of BBS proteins during development. We have previously shown that knockdown of BBS genes in the zebrafish show delayed intracellular and more recently have identified visual defects. Moreover, using a hsp70::BBS6-GFP transgenic line created in our lab, we can demonstrat rescue of knockdown phenotypes after induction of the transgene, confirming the function of our BBS6-GFP fusion protein. We next used this transgenic line to identify new proteins which interact with BBS6 in vivo. Using immunoprecitipation from transgenic BBS6-GFP embryos, candidate proteins were identified using mass spectrometry. One candidate which was identified was smarcc1a. Knockdown of smarcc1a shows defects in heart and eye development and shows a delay in intracellular transport. Smarcc1a knockdown also exacerbates the transport delay of BBS6 when sequentially injected with BBS6 MO. We have cloned out and tagged smarcc1a. We will be using this tagged form along with tagged BBS6 to confirm the interaction by co-IP in zebrafish as well as in human cell culture. The zebrafish has proven to be a powerful model system in elucidating the developmental roles for this protein complex. This work will provide insight into cilia function as well as the cellular mechanisms which underlie ciliopathies.

90. Hipk2 and PP1c-mediated dephosphorylation of Dishevelled sustains Wnt signal transduction. *Nobuyuki Shimizu, Shizuka Ishitani,* ***Tohru Ishitani****.* Medical Institute of Bioregulation, Kyushu University, Fukuoka, Fukuoka, Japan.
The Wnt family of secreted molecules controls a variety of developmental and homeostatic events. Wnt transduces its signals to downstream effectors via the phosphoprotein Dishevelled (Dvl). In the Wnt/b-catenin pathway, Dvl promotes the stabilization of b-catenin protein and consequently induces the target gene expression. In the Wnt/PCP pathway, Dvl activates the RhoA/Rac1 pathway to control cytoskeleton. Although Dvl is an essential mediator of these Wnt-mediated signaling pathways, the mechanisms that regulate the Dvl activity are not well understood. Here, we show that homeodomain-interacting protein kinase-2 (Hipk2) and protein phosphatase-1 catalytic subunit (PP1c) cooperate to regulate Dvl protein stability. First we discovered that, in zebrafish embryos, Hipk2 knockdown reduced Dvl protein and inhibited not only b-catenin pathway-dependent events but also a PCP pathway-dependent event (convergent extension). Surprisingly, Hipk2 knockdown-induced reduction of Wnt signaling was reversed by expression of Hipk2 C (the C-terminal domain of Hipk2), which doesn't include a kinase domain. In addition, in a human cell line HeLa, Hipk2 RNAi reduced Dvl stability and this reduction was also reversed by overexpression of Hipk2 C. These results suggest that Hipk2 controls Wnt signaling via its C-terminal domain in mammalian cells and zebrafish embryos. Furthermore, by biochemical analyses, we found that Hipk2 promoted both the recruitment of PP1c to Dvl and subsequent PP1c-mediated dephosphorylation of the conserved Ser/Thr residues on the Dvl C-terminal region in a kinase activity-independent manner, and that this dephosphorylation stabilized Dvl by blocking the E3 ligase Itch-mediated ubiquitination. Consistent with this finding, PP1c knockdown reduced Dvl protein levels in HeLa cells and zebrafish embryos. Moreover, co-knockdown of Itch or expression of Dvl 3A mutant, which lacks the Hipk2-PP1c dephosphorylation sites, reversed Hipk2 knockdown-induced defects in HeLa cells and zebrafish embryos. Taken together, our results suggest that Hipk2 cooperates with PP1c and counteracts Itch to maintain the level of Dvl proteins, which is required for the activation of Wnt signaling upon Wnt stimulation.

91. Regulators of host defense are critical drivers of macrophage hematopoiesis. ***Celia E Shiau****, William S Talbot.* Developmental Biology, Stanford University, Stanford, CA.
Macrophages are widely distributed innate immune cells that maintain tissue homeostasis and mediate host defense. In response to pathogen and danger signals, NOD-like receptors (NLRs) and interferon regulatory transcription factors (IRFs) act as important regulators of inflammation and immunity. Using genetic screen and advanced imaging approaches, we found that members of these families are also essential for the development of macrophages and their derivatives as tissues macrophages (such as brain microglia) in the absence of immune challenge. Our recent work has shown that macrophage formation requires suppression of inappropriate inflammatory activation by the NLR member nlrc3-like during early embryonic development, and persistent function of irf8 in allocation of progenitors to macrophage fates through mid-late larval stages. In unpublished studies our analysis of mutants in irf8 indicate an essential role for irf8 in both primitive and a period of definitive macrophage formation in zebrafish. Mutation of either gene results in complete or substantial loss of macrophages concomitant with systemic dysregulation of the innate immune system. Our results provide unique insights into the link between the regulation of immune function and normal development in macrophages.

92. Immune compromised zebrafish as a platform for cell transplantation. ***John C. Moore***[1,2], *Qin Tang*[1,2], *Nouran S. Abdelfattah*[1,2], *Jessica S. Blackburn*[1,2], *Sarah A. Martinez*[1,2], *Finola E. Moore*[1,2], *Riadh Lobbardi*[1,2], *Myron S. Ignatius*[1,2], *David M. Langenau*[1,2]. 1) Molecular Pathology, Harvard Med. School. & Mass. General Hosp. , MA; 2) Harvard Medical School and Stem Cell Institute, Boston, Massachusetts, United States of America.
Cell transplantation into immune compromised mice has revolutionized the fields of immunology, stem cell biology, regenerative medicine and serves as a critical platform for assessing therapeutic responses in vivo. However, these experiments routinely utilize small cohorts of animals, are expensive and engraftment is difficult to visualize. Capitalizing on our knowledge of which genetic mutations result in immune deficiency in mice, we have generated immune compromised strains of zebrafish. The genes targeted are key regulators of immune cell differentiation including *forkhead box N1* (*foxn1/nude*), *recombination-activating gene 2* (*rag2*), *DNA-dependent protein*

kinase (*prkdc*), and *interleukin 2 receptor gamma* (*Il2rg*). Mutations in these genes are known to cause severe combined immune deficient (SCID) phenotypes in both humans and mouse. Molecular and cellular characterization of these lines demonstrate variable immune competency with differential loss of T and B cell function. Importantly, individual models engraft stem cells from allogeneic donors including hematopoietic cells from whole kidney marrow and muscle satellite cells. Moreover, we demonstrate robust engraftment of primary or serial transplanted tumors including fluorescently labeled leukemias, muscle tumors, and melanomas from a wide range of zebrafish strains. This demonstrated ability to transplant non-immune matched cell types will revolutionize the types and scale of cell transplantation experiments performed in the zebrafish. We are currently optimizing protocols and genetic models for the xenotransplantation of human cells. The generation of immune compromised zebrafish that engraft human tissue has the potential to usher in a new era of personalized medicine, allowing for patient stratification into open clinical trials based on responses to therapy in the zebrafish xenograft model and for novel drug discovery.

93. Cxcr3/Cxcl11 Signaling Mediates Macrophage Activation and Chemoattraction to Bacterial Infection in Zebrafish. ***Vincenzo Torraca***[1], *Chao Cui*[1], *Ralf Boland*[1], *Jan-Paul Bebelman*[2], *Marco Siderius*[2], *Martine J. Smit*[3], *Astrid M. van der Sar*[4], *Herman P. Spaink*[1], *Annemarie H. Meijer*[1]. 1) Institute of Biology, Leiden University, Leiden, The Netherlands; 2) Department of Biochemistry and Molecular Biology, VU University, Amsterdam, The Netherlands; 3) Division of Medicinal Chemistry, VU University, Amsterdam, The Netherlands; 4) Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands.
The recruitment of macrophages to infectious foci depends widely on the local release of chemoattractant mediators. However, the exact signals mediating this response and the relevance of these signaling cues *in vivo* remain poorly defined. Using non-invasive imaging in whole zebrafish embryos, we examined the role of the Cxcr3/Cxcl11 axis in macrophage recruitment to localized bacterial infections. In a *null* mutant of *cxcr3.2*, macrophage chemotaxis to bacterial infection was significantly attenuated, while recruitment to other chemoattractant stimuli (fMLP, LTB4 and chemically-induced inflammation) was not altered. With the aim of de-orphanizing Cxcr3.2, we selected infection-inducible candidate ligands from a cluster of putative CXCL11-like chemokine genes. Recombinant chemokines Cxcl11aa and Cxcl11af exhibited a macrophage-specific and Cxcr3.2-dependent chemoattraction when locally administrated to embryos. On the contrary, a third ligand, Cxcl11ae, induced promiscuous neutrophil/macrophage recruitment which was not abolished by *cxcr3.2* loss-of-function. Moreover, Cxcr3.2 deficiency significantly affected the expansion and seeding of *Mycobacterium marinum* granulomatous lesions. These findings demonstrate the existence of a functional Cxcr3/Cxcl11 signaling axis in zebrafish and highlight its crucial role in guiding the macrophage response to infection. Additionally, we show that abolishment of Cxcr3-dependent recruitment is beneficial to restrict macrophage-mediated mycobacterial dissemination, thereby providing a valuable model to study the effects of manipulation of the Cxcr3/Cxcl11 axis in the establishment of mycobacterial infections.

94. Dose-dependent virulence changes following localized infection in zebrafish embryos with a Listeria monocytogenes strain designed to hyper-activate the Nlrc4 inflammasome. ***William JB Vincent***[1], *John-Demian Sauer*[1], *Anna Huttenlocher*[1,2]. 1) Microbiology Doctoral Training Program, Dept. of Med. Microbiology & Immunology, University of Wisconsin-Madison, Madison, WI; 2) Dept. of Pediatrics, University of Wisconsin-Madison, Madison, WI.
The inflammasome is a protein complex that forms upon the sensing of intracellular danger and pathogen associated molecular patterns leading to the rapid induction of inflammation and cell death. However, the role of the inflammasome in coordinating the response to infection is still largely uncharacterized. Furthermore, while characterization of human and mouse inflammasomes has been advanced in recent years, little is known about the presence of these complexes in zebrafish. The intracellular bacterial pathogen, Listeria monocytogenes, has been experimentally shown to activate several inflammasome complexes, however in vivo this activation is highly regulated by the bacterium and activation is likely avoided by most intracellular bacterial pathogens. To examine the presence and function of inflammasome complexes in the zebrafish, we compared the host response to infection with wild type L. monocytogenes and a strain engineered to express the Nlrc4 inflammasome agonist, Legionella pneumophila flagellin (LpFla). Apoptosis-associated speck-like protein containing a CARD (ASC), an adaptor of many inflammasomes, forms visible specks thought to be the sight of inflammasome formation. Activation of the inflammasome in zebrafish cells is supported by an increase in ASC specks following LpFla infection. As observed in the mouse model of listeriosis, LpFla is highly attenuated following infection at WT LD50 doses. Surprisingly, at a very high inoculum LpFla causes rapid, increased death, suggesting that this phenotype is dependent on a recently described pathway downstream of the Nlrc4 inflammasome leading to uncontrolled eicosanoid production. In support of this, treatment with a cycloxygenase inhibitor reduces the rapid death following high-dose LpFla infection. Together these studies define the presence of the Nlrc4 inflammasome in zebrafish and establish that inflammasome function is beneficial or detrimental to host responses depending on the extent of activation.

95. Epigenetics Regulation of Intestinal Inflammation in Zebrafish. *Lindsay Marjoram*[1], *Ashley Alvers*[1], *Elizabeth Deerhake*[2], *Jennifer Bagwell*[1], *Jamie Mankiewicz*[3], *Rebecca Beerman*[4], *Jason Willer*[5], *Nicholas Katsanis*[5], *David Tobin*[4], *John Rawls*[4], *Mary Goll*[2], ***Michel Bagnat***[1*]. 1) Department of Cell Biology, Duke University Medical Center, Durham, NC 27710; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10065; 3) Department of Biology, North Carolina State University, Raleigh, NC 27695; 4) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710; 5) Center for Human Disease Modeling, Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.
In a forward genetic screen carried out in our lab, we identified mutants with defects in intestinal epithelial integrity. One of these mutants,*aa51.3*, shows excess epithelial cell shedding and apoptosis that is accompanied by a loss of intestinal barrier function and neutrophil infiltration. These defects are consistent with hallmarks observed in inflammatory bowel disease (IBD). IBD is tightly associated with high levels of expression of tumor necrosis factor (Tnf), a pro-inflammatory cytokine that plays a prominent role in intestinal inflammation and the loss of barrier function in IBD. To monitor Tnf expression, we generated an inflammation-responsive tumor necrosis factor transgenic line, *TgBAC(tnfa:GFP)*. In *aa51.3* mutants, *TgBAC(tnfa:GFP)* is dramatically upregulated in the epithelium along the

entire length of the intestine. Elevated *TgBAC(tnfa:GFP)* is influenced in part by microbiota colonization and is associated with increased recruitment of neutrophils and barrier defects in the intestine. Interestingly, tnfa knockdown rescued the intestinal epithelial integrity and barrier defect phenotypes in *aa51.3* mutants. Exome sequencing followed by positional cloning identified a causative splice-site mutation in a gene that plays a fundamental role in epigenetic regulation of gene expression. Our data reveal that loss of *tnfa* regulation in the intestine leads to a microbiota-dependent IBD-like phenotype.

96. Chemical screen shows link between b-catenin and the c-Jun-N-terminal kinase pathway in liver tumor formation. ***Kimberley Evason***[1], *Andrei Goga*[2], *Didier Stainier*[3]. 1) Department of Pathology, UCSF, San Francisco, CA; 2) Department of Cell and Tissue Biology, UCSF, San Francisco, CA; 3) Max Planck Institute for Heart and Lung Research, Dept. III - Developmental Genetics, Bad Nauheim, Germany.
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Mutations in the gene encoding b-catenin, part of the Wnt signaling pathway, define a major subset of HCC and may represent an early or initiating event in these tumors. However, there are no clinically approved drugs that target the Wnt/b-catenin pathway. In mice, expression of constitutively active b-catenin is not sufficient for HCC formation, limiting the utility of mouse models in studies of b-catenin-related liver tumorigenesis. We found that transgenic adult zebrafish expressing hepatocyte-specific activated b-catenin (*Tg(fabp10a:pt-b-cat)* fish) have decreased survival and enlarged livers with histologic changes resembling human HCC. Furthermore, liver enlargement in *Tg(fabp10a:pt-b-cat)* larvae is easily appreciated by 6 days post fertilization. We used these animals to screen for druggable pathways that mediate b-catenin-induced liver growth and identified two c-Jun N-terminal kinase (JNK) inhibitors that suppressed this phenotype. Activated b-catenin was associated with JNK pathway activation in zebrafish larvae and adult livers. We also found that human HCCs with b-catenin activation showed greater *JUN* expression than HCCs without b-catenin activation, underscoring the relevance of our zebrafish studies to human HCC. Our results implicate the JNK pathway as a mediator of b-catenin-induced liver tumor formation and raise the possibility that JNK inhibitors represent potential treatments for a major subgroup of HCC.

97. Antioxidant Treatment Rescues Hematopoietic Phenotypes in Different Models of Adenylate Kinase 2 Deficiency. ***Alberto Rissone***[1], *Katja Weinacht*[3], *Jaya Jagadeesh*[1], *Karen Simon*[1], *Kevin Bishop*[2], *Raman Sood*[2], *Luigi Notarangelo*[4], *Fabio Candotti*[1]. 1) Genetics and Molecular Biology Branch, NHGRI - National Institutes of Health, Bethesda, MD; 2) Zebrafish Core Facility, GMBB, NHGRI - NIH, Bethesda, MD; 3) Division of Hematology/Oncology, Children's Hospital Boston, Boston, MA; 4) Division of Immunology, Children's Hospital Boston, Boston, MA.
Adenylate kinase 2 (AK2) is a mitochondrial enzyme that plays a critical role in cellular energy homeostasis. In humans, mutations in the AK2 gene cause reticular dysgenesis (RD), one of the most profound forms of severe combined immunodeficiency (SCID). RD is characterized by the combined lack of granulocytes and lymphocytes, leading to early, recurrent and overwhelming infections. Currently, hematopoietic stem cell transplant (HSCT) is the only available treatment for RD. The mechanisms underlying the pathophysiology of RD remain unclear. We generated two zebrafish models using zinc finger nuclease-mediated ak2 gene knock-out and ENU-induced mutants. We found that the ak2 deficiency results in severe impairment of hematopoietic stem cells development, which results in strong defects in the erythroid, myeloid and lymphoid hematopoietic lineages. We also observed increased levels of reactive oxygen species triggering oxidative stress and apoptosis in ak2-deficient embryos. Importantly, antioxidant treatment with N-acetyl-L-cysteine and Glutathione resulted in the rescue of oxidative stress and, more importantly, of the hematopoietic phenotypes in both our zebrafish mutants. To assess whether these results may be translated into new therapeutic approaches for RD, we generated induced pluripotent stem cells (iPSCs) derived from fibroblasts of an AK2-deficient patient and verified that in vitro granulocytic differentiation of these cells is blocked at the promyelocyte stage, similar to what observed in RD patients bone marrow. Consistent with our results in ak2-mutant zebrafish, exposure of AK2-deficient iPSCs to Glutathione restored granulocyte differentiation, thus supporting the mechanistic hypothesis involving abnormal redox state in AK2 deficiency and providing potential therapeutic avenues for patients affected with RD.

98. A chemical suppressor of *tif1g* mutant *moonshine* reveals that PPARa bypasses the transcriptional pausing defect in erythroid differentiation. ***Marlies P. Rossmann***[1], *Xiaoying Bai*[2,3], *Bilguujin Dorjsuren*[1], *Lauren Krug*[1], *Chang-Ying Chiang*[1], *Erika Lee*[1], *Wahaj Chaudhry*[1], *Shakkaura Kemet*[1], *Isaac Adatto*[1], *Leonard I. Zon*[1,2]. 1) Department of Stem Cell and Regenerative Biology , Harvard University, Cambridge, MA; 2) Howard Hughes Medical Institute, Boston Children's Hospital, Harvard Medical School, Boston, MA; 3) Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center, Dallas, TX.
Aberrant erythropoiesis results in anemias and leukemias and can be caused by dysregulated transcription elongation mechanisms at erythroid loci at specific differentiation steps. Transcriptional intermediary factor 1 gamma (*TIF1g*), the gene mutated in the bloodless zebrafish *moonshine* mutant, recruits positive elongation factors to erythroid genes to relieve paused Pol II. To elucidate *TIF1g*-mediated mechanisms in erythroid differentiation, we have performed a chemical suppressor screen in *moonshine* mutant embryos testing 4,000 chemical compounds. Using this strategy we have identified peroxisome proliferator-activated receptor alpha (PPARa) agonists, most importantly, Clofibrate to rescue *be3 globin*-expression in 70-90% of *moonshine* embryos. Interfering with PPARa using morpholinos or the PPARa antagonist GW6471 significantly reduced this rescue. To identify the PPARa-interacting proteome in an erythroid progenitor context, we established a human K562 erythroleukemia cell line with doxycyclin-inducible expression of Flag-PPARa and PPARa target genes. Large-scale Flag-immunoprecipitation followed by mass spectrometric analysis identified PPARa's heterodimerization partner RXR, co-activators and -repressors, 24 subunits of the mediator complex, 6 of the cohesin complex, 7 RNA Pol II subunits as well as DSIF and PTEF-b, 2 proteins with a positive role in transcription elongation. Given the association with specific elongation and chromatin factors, PPARa likely integrates these factors to facilitate elongation at erythroid genes. Our studies provide a basic understanding of the processes regulating transcription elongation in the differentiation of hematopoietic cells, and could lead to novel therapeutic strategies.

99. Interception of host angiogenic signalling limits mycobacterial growth. ***Stefan H Oehlers***[1], *Ninecia R Scott*[1], *Monica I Thomas*[1], *Kazuhide S Okuda*[2], *Mark R Cronan*[1], *Philip S Crosier*[2], *David M Tobin*[1]. 1) MGM, Duke University Medical Center, Durham, NC; 2) Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand.
Pathogenic mycobacteria induce the formation of complex cellular aggregates called granulomas that are the hallmark of tuberculosis. Here we examine the development and consequences of vascularization of the tuberculous granuloma, using intravital microscopy in a zebrafish-Mycobacterium marinum infection model that closely resembles human tuberculosis. We show that angiogenesis is intimately associated with granuloma formation. The initiation of angiogenesis at the granuloma coincides with the generation of local hypoxia and transcriptional induction of the canonical pro-angiogenic molecule VEGFA. Pharmacological inhibition of the VEGF pathway suppressed granuloma-associated angiogenesis, reduced infection burden and limited dissemination. Moreover, anti-angiogenic therapies synergised with the first-line anti-tubercular antibiotic rifampicin as well as with the antibiotic metronidazole, which targets hypoxic bacterial populations. Our data suggest that mycobacteria induce granuloma-associated angiogenesis, which promotes mycobacterial growth and increases spread of infection to new tissue sites. We propose the use of anti-angiogenic agents, now being used in cancer regimens, as a host-targeting TB therapy, particularly in extensively drug-resistant disease where current antibiotic regimens are largely ineffective.

100. Genetic analysis of the acoustic startle behavioral threshold. ***Kurt Marsden***[1], *Roshan Jain*[1], *Marc Wolman*[2], *Hannah Bell*[1], *Lauren Schmidt*[1], *Julianne Skinner*[1], *Katharina Hayer*[1], *John Hogenesch*[1], *Michael Granato*[1]. 1) University of Pennsylvania School of Medicine, Philadelphia, PA; 2) University of Wisconsin, Madison, WI.
A critical function of the nervous system is to determine whether a stimulus requires a behavioral response. For example, an appropriate threshold must be set such that only loud auditory stimuli that indicate danger will induce a startle response. Dysregulation of the startle response is observed in many neuropsychiatric disorders including schizophrenia, autism, and anxiety disorders. To identify molecular-genetic pathways that regulate the startle threshold and its modulation we used the zebrafish model system to conduct a forward genetic screen for hypersensitive startle phenotypes in which mutant larvae startle in response to low-level acoustic stimuli that fail to startle wild-types. We have isolated 9 mutants in this class, and further analysis of two of these, *whisper2000* and *escapist*, has revealed that the startle threshold can be regulated at multiple levels within the circuit. Hypersensitivity of *whisper2000* but not *escapist* mutants is abolished by ablation of lateral line neuromasts. Furthermore, in a pre-pulse inhibition assay to identify the hearing detection threshold, *whisper2000* mutants display enhanced hearing while *escapist* mutants do not, indicating that in *escapist* mutants the defect is startle specific. Thus, startle sensitivity can be set at the level of sensation or at the input to the hindbrain startle circuit. Using whole-genome sequencing we have identified the causative mutation in another hypersensitive mutant, *06JRKS*, as a premature stop codon in the gene *cyfip2* (cytoplasmic FMR1 interacting protein 2). *cyfip2* is dysregulated in Fragile X syndrome and is known to be critical for axon guidance and synaptogenesis. Current work on $cyfip2^{06JRKS}$ is focused on identifying the origin of startle hypersensitivity using simultaneous Ca^{2+} and behavioral imaging, examining synapse numbers and morphology, and confirming the cell-type requirements for *cyfip2*. In combination, these studies of startle hypersensitivity will establish mechanisms that regulate the acoustic startle threshold and thereby illuminate disease processes in conditions such as anxiety and autism.

101. High-throughput Targeted Mutagenesis using CRISPR-Cas9 in Zebrafish. ***Gaurav K. Varshney***[1], *Wuhong Pei*[1], *Johan Ledin*[2], *Matthew LaFave*[1], *Jennifer Idol*[1], *Viviana Gallardo*[1], *Lisha Xu*[1], *Marypat Jones*[3], *Ursula Harper*[3], *Blake Carrington*[4], *Kevin Bishop*[4], *Meghana Vemulapalli*[5], *Mingyu Li*[6], *Wenbiao Chen*[6], *James Mullikin*[5], *Raman Sood*[4], *Shawn M Burgess*[1]. 1) Translational and Functional Genomics Branch, National Human Genome Research Institute, NIH, Bethesda, MD, USA; 2) Department of Organismal Biology, Uppsala Universitet, Uppsala, Sweden; 3) Genomics Core, NHGRI, NIH, Bethesda, MD; 4) Zebrafish Core, NHGRI,NIH, Bethesda, MD; 5) NIH Intramural Sequencing Center, NHGRI, NIH, Bethesda, MD; 6) Vanderbilt University, Nashville, TN.
The zebrafish genome is now complete and is only the third vertebrate to have a fully annotated reference genome, which facilitates systematic large-scale functional genomic studies. The number of large-scale mutagenesis projects has increased in last few years, further enhancing the utility of zebrafish as a model organism. Most of these projects use random mutagenesis approaches thus limiting the number of genes that can be mutagenized with this approach in a cost-effective manner. However, the development of targeted mutagenesis approaches such as TALENs and CRISPR-Cas9 have opened up new avenues to mutagenize genome in a systematic fashion. The bacterial derived RNA-guided Cas9 endonuclease has emerged as a very powerful genome-editing tool in a wide variety of cells and organisms. We developed an inexpensive high-throughput method of multi-allelic targeted mutagenesis using the CRISPR-cas9 system. As a proof-of-principle, we targeted over 400 genes that included all zebrafish known or candidate orthologs for deafness in humans, genes known to be involved in lateral line migration, selected kinase genes, and many genes involved in proteoglycan synthesis. By designing two targets per gene and using a high-throughput fluorescent PCR approach, we easily identified mutations at both targets individually as well as deletions of the regions between targets. We also generated a highly fecund lab strain NHGRI-1 and mapped all the possible polymorphisms in this line by deep sequencing. By having all polymorphisms identified, it allows us to computationally design CRISPR targets without additional target sequence validation. All these mutants as well as NHGRI-1 will be released to the community through ZIRC.

102. GAPmap: Small Meiotic Linkage Groups to Fix Gaps in the Zebrafish Reference Genome Assembly. ***Derek L Stemple***, *Ian Sealy*, *Ross N. W. Kettleborough*, *James Torrance*, *Kerstin Howe.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA United Kingdom.
The Zv9 assembly of the zebrafish genome was anchored by a high-density, high-resolution meiotic map call SATmap. In SATmap, unfortunately about 10% of the genomic regions were found to be monomorphic and thus did not provide information to place sequence contigs. These monomorphic regions, were nevertheless anchored with lower resolution MGH and HS map markers, which allowed sparse placement of contigs, but often orientation of these contigs was found to be ambiguous. To fill-in the gaps we devised a new strategy. We capitalized on the MGH cross, pull-down reagents and Illumina sequencing to generate small, high-resolutions meiotic linkage groups to

spanning the gaps in SATmap.

We used genomic DNA from 190 F2 fish from the MGH cross, as the source material. This cross was generated independently of the SATmap cross and was made from an otherwise wild-type AB strain zebrafish and an India strain zebrafish as grandparents.

We obtained the collection DNA from the F2 fish. These DNAs were used to make indexed Illumina libraries, enriched for the gap regions and sequenced. Sequences were mapped against the Zv9 assembly and single-nucleotide polymorphisms were called. These polymorphisms were then used to generate a set of new small gap-spanning linkage groups. We identified more than 20,000 new markers and were able to place a significant fraction of previously unplaced sequence into chromosomes.

103. A simple method for targeted insertions into zebrafish using TALENs. ***Sean Constable***[1], *Sebastian Gerety*[2], *David Wilkinson*[1]. 1) MRC National Institute for Medical Research, London, United Kingdom; 2) Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
The recent advent of targetable nuclease technology such as TALENs has provided us with the ability to induce double stranded breaks within the genome with a high degree of precision and efficiency. There have been several successful reports describing the insertion of exogenous sequences into target locations in the zebrafish genome through homologous recombination, but routine use of this method is hampered by low efficiency of the insertion event. Here we describe a simple method to efficiently insert custom DNA sequences into precise locations in the zebrafish genome that doesn't rely on sequence homology but instead involves a donor plasmid containing the TALEN binding site upstream of the insertion cassette. We inject this donor plasmid in combination with RNA encoding the TALEN pair into zebrafish embryos in order to insert fluorescent proteins and GAL4 into regions associated with endogenous promoters, generating reporter lines that reflect the expression pattern of those genes. Over 75% of injected embryos show evidence of insertion and by selecting embryos that contain the highest amount of insertion we find that 27% of these fish transmit the insertion to their progeny. The design of the donor plasmid is such that simple cloning strategies can be used to rapidly change both the target site and the insertion cassette to that of the user's choice. This simple and efficient technique adds to the growing repertoire of tools for manipulating the zebrafish genome.

104. Advances in Gene-Targeting in the Zebrafish. ***Kazuyuki Hoshijima***, *Mick Jurynec, David Grunwald*. Human Genetics, University of Utah, Salt Lake City, UT.
Gene-targeting, the replacement of specific genomic sequences with exogenously provided sequences, allows for designed modifications of the genome. In yeast and mouse, gene-targeting via homologous recombination (HR) has served as a powerful strategy for eliminating gene function, controlling gene expression conditionally, or visualizing cells that expressed an endogenous gene. As HR events are stimulated by Double Strand Breaks (DSBs) in host chromosomes, the recent development of highly efficient engineered nucleases including ZFN, TALEN and CRISPR raised the possibility of extending gene-targeting via HR to new organisms. Here we describe generation of four types of designed modifications of the zebrafish genome, demonstrating that gene-targeting can be readily accomplished in the zebrafish. Gene-targeting was accomplished by injecting zebrafish fertilized eggs with TALEN mRNA to induce targeted DSBs and donor dsDNA with homology to the targeted locus. In a manner dependent on TALEN activity, we achieved: 1) accurate sequence modifications of a targeted locus, 2) sequence modifications accompanied by the concurrent nearby integration of a reporter gene used to mark acquisition of the modification, 3) 'knock-in/knock-out' mutations in which host gene function is destroyed and GFP reporter sequences are productively expressed from the targeted locus; and 4) in-frame insertion of sequences encoding antigen peptides resulting in the generation of viable epitope-tagged alleles of an endogenous gene product. All gene-targeting events were stable and heritable, and integration efficiencies were high enough to establish gene-targeted fish lines. Thus, gene-targeting stimulated by targeted DSBs is now feasible in zebrafish, and our approach should also be applicable in other model organisms.

105. The Zebrafish Mutation Project: New Mutagenesis and Phenotyping Strategies. ***Elisabeth M. Busch-Nentwich***, *John Collins, Neha Wali, Ian Sealy, Peter Clarke, Christopher M. Dooley, Catherine Scahill, Richard J. White, Nicole Staudt, Samantha Carruthers, Jorge Zamora, Zsofia Pusztai, Isabel Brocal, Amanda Hall, Richard Clark, Richard Gibbons, Ross N.W. Kettleborough, Derek L. Stemple.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
With over 60% of zebrafish protein coding genes targeted through random ENU mutagenesis the Zebrafish Mutation Project (ZMP) is now at a juncture where CRISPR directed mutation generation increasingly supplements and will eventually replace ENU mutagenesis. Among other efficiency based considerations, this shift to targeted disruption is driven by the identification of novel genes of interest through our transcriptional profiling of mutants. DeTCT (Differential expression Transcript Counting Technique) is a sequencing based analysis method comparable to RNASeq, with the important distinction that it is restricted to the quantification of 3'ends of mRNA. Our technique allows us to simultaneously analyse the genotypic profile and global mRNA expression of individual embryos. This gives us access to transcriptional changes that precede the emergence of morphological phenotypes, thus enabling us to construct directional gene regulatory networks *in vivo* on an unprecedented scale. Furthermore, the sensitivity is such that transcriptional changes even in rare cell types can be detected reliably within the whole embryo. I will present how the morphological and molecular analysis of mutants and different developmental stages connects previously uncharacterised genes to diverse developmental processes, and identifies unannotated transcripts and their biological function.

106. Epigenetic therapy restores normal hematopoiesis in a zebrafish model of NUP98-HOXA9-induced myeloid disease. ***Adam Deveau***[1,2], *A. Micheal Forrester*[1,2], *Andrew Coombs*[1,3], *Gretchen Wagner*[1,3], *Clemens Grabher*[6,7], *Ian Chute*[5], *Daniel Leger*[5], *Stephen Lewis*[2,4,5], *A. Thomas Look*[6], *Jason N. Berman*[1,2]. 1) Pediatrics, IWK Health Centre, Halifax, NS, Canada; 2) Departments of Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada; 3) Department of Marine Biology, Dalhousie University, Halifax, NS, Canada;

4) Atlantic Cancer Research Institute, Dalhousie University, Halifax, NS, Canada; 5) Department of Biology, University of New Brunswick, Saint John, NB, Canada; 6) Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 7) Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Karlsruhe, Germany.
Acute myeloid leukemia (AML) occurs when multiple genetic aberrations alter white blood cell development, leading to hyperproliferation and arrest of cell differentiation/maturation. Pertinent animal models serve as a critical link between in vitro studies to the use of new agents in clinical trials. We previously expressed human NUP98-HOXA9 (NHA9), a fusion oncogene found in high-risk AML, in zebrafish and produced a pre-leukemic state where embryos demonstrated myeloid expansion at the expense of erythroid development, and adult fish developed a myeloproliferative neoplasms (MPN) (Forrester et al BJH 2011).. We have now leveraged this model to show that NHA9 acts at the level of the hematopoietic stem cell, and that the oncogenic activity of NHA9 depends on overexpression of meis1, the Wnt/b-catenin pathway, and a novel downstream factor dnmt1 - a DNA methyltransferase that represses myeloid development through epigenetic regulation. Normal hematopoiesis was restored in NHA9 embryos with knockdown of meis1 or dnmt1, as well as pharmacologic treatment with DNMT inhibitors or COX inhibitors. Strikingly, we discovered synergy when we combined sub-monotherapeutic doses of a histone deacetylase inhibitor plus either a DNMT inhibitor or COX inhibitor to block the effects of NHA9 on zebrafish blood development. Our work identifies druggable targets in NHA9-induced myeloid disease, and suggests new rational treatment strategies by combining minimal doses of known bioactive compounds.

107. Mutation of kri1l causes ribosomopathy-like hematopoiesis failure via excessive autophagy. *Xiaoe Jia[1], Ke Ma[1], Chunguang Ren[1], Changbin Jing[1], Cong Fu[1], Wenjuan Zhang[1], Shuang Wu[1], Yi Chen[2], Yi Jin[2], Mei Dong[1], Min Deng[1], Lingfei Luo[4], Li Li[4], Yi Zhou[3], Leonard Zon[3], Saijuan Chen[2], **Weijun Pan**[1*]*. 1) Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences and Graduate University, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, China China; 2) State Key Laboratory for Medical Genomics, Shanghai Institute of Hematology, RuiJin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 3) Stem Cell Program, Hematology/Oncology Program at Children's Hospital Boston, Harvard Medical School, Boston, MA02114, USA; 4) Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, Laboratory of Molecular Developmental Biology, School of Life Sciences, Southwest University, Beibei, Chongqing, China.
Dysregulation of ribosome biogenesis called ribosomopathies causes human diseases, such as Diamond-Blackfan anemia (DBA) or 5q minus (5q-) syndrome. The mechanism of the blood disorder in these diseases remains elusive. Here, through characterization and cloning of a novel zebrafish mutantLDD499, we report a novel connection between excessive autophagy and ribosomal dysfunction in hematopoietic stem and progenitor cells (HSPCs). This mutant carries a recessive lethal mutation in kri1l gene-an essential component of rRNA small subunit processome. Kri1l function is required for normal ribosome biogenesis and expansion of definitive HSPCs and subsequent lineage differentiation. Overexpression of bcl2, but not p53 inhibition, can fully rescue the hematopoietic defects in kri1lLDD499 mutants. This rescue is distinguished from cell apoptosis regulated by p53 in other ribosomopathies, Through live imaging and biochemical studies, we found loss of kri1l induced excessive autophagy in HSPCs, and treatment of autophagy inhibitors (3-MA, Baf A1) could markedly prolong HSPCs lifespan and recover all hematopoietic lineages in kri1lLDD499 mutants. A subset of ribosomopathies induces autophagy and inhibition of autophagy by small molecules may be effective as potential therapy for these disorders.

108. FGF signaling specifies hematopoietic stem cells through its regulation of somitic Notch signaling. ***Y. Lee***[1], *J. Manegold[1], A. Kim[1], D. Stachura[1], W. Clements[1,2], D. Traver[1]*. 1) Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; 2) Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN.
Hematopoietic stem cells (HSCs) are rare cells with the ability to both self-renew and regenerate all mature blood cell types over the lifespan of an organism. Recent studies have conclusively demonstrated that HSCs arise from hemogenic endothelium, a special population of endothelial cells within the ventral wall of the dorsal aorta that transdifferentiate into HSCs. Development of HSCs requires complex interactions between a diverse number of molecular signaling pathways and downstream intracellular transduction networks. Previous studies suggested that one group of signaling molecules, the fibroblast growth factors (FGFs), regulate hematopoiesis in vertebrates. However, the role of FGF signaling in HSC development has not been addressed. Here, we examined the role of Fgf signaling in HSC development using transgenic zebrafish embryos and found that FGF signaling is required to specify HSCs in a non-cell-autonomous manner during mid-somitogenesis stages. We previously identified a novel Wnt16-Notch signaling pathway that acts within the somite to relay required specification signals to HSC precursors by regulating the expression of two Notch ligands, deltaC (dlc) and deltaD (dld), whose combined function is essential for HSC fate. Our current studies demonstrate that FGF signaling, via FGF receptor 4 (Fgfr4), mediates a signal transduction pathway between wnt16 and dlc, but not dld, to regulate HSC emergence. Taken together, our findings demonstrate that FGF signaling is required in the developmental HSC niche to relay non-canonical Wnt16 activity to the Notch pathway to specify HSC fate.

109. Role of tp53 in the oxidative stress response of erythroid precursors. ***Michelle Carter**, Richard Shimshock, Ashley Kramer, Troy Lund.* University of Minnesota, Minneapolis, MN.
Oxidative stress plays a key role in acute and chronic anemia. Erythroid precursors and mature red cells have increased sensitivity to oxidative stress. As many of the central genes in this pathway are necessary for life, it has been difficult to create animal models to explore genetic components. Our zebrafish model permits pro-oxidant exposure early in hematopoietic development, and *gata1*^{DsRed} transgenic animals allow clear identification of erythroid precursors, allowing us to examine the specific effects of oxidative stress on these cells. After 72 hours of exposure to the strong pro-oxidant naphthol, embryonic zebrafish up-regulated anti-oxidant genes including *hif1a*, *nrf2*, *fth1a*, *txn*, and *hmox1*. Promoter analysis revealed tp53 binding sites within 4 kb of the first exon in each gene. The tumor-suppressor protein tp53 is thought to act primarily as a transcription factor; mutations in *tp53* are found in more than 50% of all human tumors. We

showed by qRT-PCR that naphthol induced *tp53* expression in zebrafish embryos 3-fold over baseline. The zebrafish mutant *tp53*^{M214K} contains a point mutation in the DNA binding region of *tp53* eliminating this activity; the corresponding mutation has been identified in human tumors. Compared to wild-type, *tp53*^{M214K} embryos were highly sensitive to pro-oxidants, showing a 3.2-fold increase in anemia and cardiac edema; a 3-fold decrease in the number of hemoglobin-staining cells; and a doubling of generated ROS. In contrast, reduction of tp53 protein by morpholino knockdown, or inhibition of tp53 activity by treatment with pifithrin, resulted in reduction of generated ROS after naphthol treatment. We hypothesize that disregulation of DNA-binding activity by tp53 leads to an increase in ROS production, while elimination of tp53 protein is protective against formation of ROS. Understanding the mechanisms by which the anti-oxidant response is regulated will assist in the discovery of more effective drug-able targets to treat oxidative stress accompanying acute and chronic anemia.

110. Advantages of Clonal Zebrafish for Transplantation Assays and Investigation of the Immune System. ***Sean C. McConnell***[1], *Wilfredo Marin*[1], *Michael Peters*[1,2], *Jill L.O. de Jong*[1]. 1) Department of Pediatrics, Section of Hematology-Oncology and Stem Cell Transplant, University of Chicago, Chicago, IL 60637; 2) Department of Biology, Northeastern University, Boston, MA 02115.
The use of isogenic animals provides a defined genetic background that minimizes unpredictable phenotypic variation, improves the power of mechanistic studies, and also advances new lines of investigation, for example, transplantation immunology. Isogenic mouse strains have been used for decades, but isogenic zebrafish lines have been difficult to maintain due to severe inbreeding depression, which is associated with reduced fecundity, sex ratio bias, and increased susceptibility to common diseases. Recently, Revskoy and colleagues generated several stable homozygous diploid lines, including CG1 and CG2, using parthenogenesis. We selected the CG2 clonal line for our experiments and have bred and raised these fish to successfully maintain them in large numbers. Here we show that clonal CG2 zebrafish are amenable to stable transgenesis using the Tol2 system. We next employ transgenic ubb:GFP clonal lines as marrow donors to perform fully immune-matched hematopoietic transplantation assays for studies of hematopoietic stem cell function. We also use these fish to investigate mutant gene function in hematopoietic stem cells using competitive repopulation assays. Finally, we characterize the unusual MHC class I core locus of the CG2 clonal line, which contains distinct isoforms of antigen processing genes compared with those found in other fish strains. These studies provide an important foundation for advancing zebrafish as a disease model, and facilitate future experiments that rely on transplantation assays in adult animals. In conclusion, clonal zebrafish provide many advantages over outbred fish, and we anticipate that these lines will gain more widespread use particularly for transplantation assays and experiments to define the immune system.

111. Characterizing the Roles of Sox2 and Sox3 in Sensory/Neural Patterning During Zebrafish Inner Ear Development. ***Yunzi Gou***, *Hye-Joo Kwon, Bruce Riley.* Texas A&M University, college station, TX.
Vertebrate Sox2 and Sox3 are closely related members of the SoxB1 transcription factor family involved in early neural development and stem cell maintenance. Previous studies suggest that functional diversity of SoxB1 factors depends on their level of expression and availability of other cofactors. We are exploring these parameters in the context of inner ear development. Zebrafish *sox2* and *sox3* are expressed in partially overlapping domains in the otic placode and in the floor of the otic vesicle. We hypothesize that Sox2 and Sox3 help promote sensory vs. neurogenic development in abutting spatial domains within the otic vesicle. Expression of *sox2* normally marks developing sensory epithelia in a medial domain, and knockdown of *sox2* reduces production and maintenance of hair cells. In contrast, although expression of *sox3* marks both sensory and neural precursors, knocking down *sox3* reduces neurogenesis but does not affect hair cell production. High-level misexpression of either Sox2 or Sox3 greatly expands both sensory and neurogenic domains. The medial factor Pax2a is specifically required for Sox2/3 to expand the sensory epithelia, but not the neurogenic domain, identifying at least one cofactor that influences SoxB1 function. Additionally, the concentration of Sox2 (but not Sox3) critically affects function: moderate misexpression of Sox2 inhibits neurogenesis while promoting sensory development, revealing a fundamental difference between Sox2 and Sox3. Thus both the level of expression and availability of regionally expressed cofactors diversify the functions of Sox2 and Sox3 and helps coordinate sensory and neural patterning in the inner ear.

112. Neurogenin1 regulates the projection of axons of olfactory neurons via its target cxcr4b. *Romain Madelaine, Raphael Aguillon, Laurence Garric, Caroline Halluin, Pascale Dufourcq,* ***Patrick Blader***. Centre de Biologie du Developpement, CNRS/Universite Paul Sabatier, Toulouse, France.
It is well established that bHLH proneural transcription factors control the expression of targets that are required for neural determination. It has also been suggested that proneural factors regulate sets of genes involved in various aspects of neural specification and cell behaviour. Indeed, the Atonal family member Neurogenin 2 (Neurog2) has recently been shown to control radial migration of newly formed cortical neurons via direct transcriptional regulation of the small GTP-binding protein Rnd2. We have shown that neurogenesis in the zebrafish olfactory placode is delayed in neurogenin 1 (neurog1) mutants. While neurogenesis partially recovers in the absence of Neurog1, we show here that residual olfactory neurons that form in the mutant fail to project correctly. We show that a known regulator of olfactory neuron projections in the zebrafish, cxcr4b, is a transcriptional target of Neurog1. Furthermore, reintroduction of cxcr4b expression in olfactory neuron progenitors in the neurog1 mutant efficiently rescues the projection phenotype. Finally, confocal analysis of the behaviour of single cell clones suggests that the failure of olfactory neurons to project is due to a loss of cell polarity during olfactory placode morphogenesis. We conclude that Neurog1/Cxcr4b are required to prefigure the site from which olfactory sensory neurons will emerge from the olfactory placode before projecting to the olfactory bulb.

113. Assaying retinal circuit function and vision in the retina lacking Müller glia cells. ***Ryan MacDonald***[1], *Nachiket Kashakar*[2], *Jingjing Zang*[3], *Stephan Neuhauss*[3], *Leon Lagnado*[2], *William A. Harris*[1]. 1) Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom; 2) School of Life Sciences, University of Sussex, United Kingdom; 3) Institute of Molecular Life Sciences, University of Zurich, Switzerland.
Müller glia (MG) are the lone glial cell type born within the retina. MG extend elaborate processes into the synaptic layers of the retina and are thought to have a myriad of important functions, including retinal synaptogenesis, recycling of neurotransmitters and maintaining homeostasis. We have developed a method to completely block all MG from specifying in the embryonic zebrafish retina. Surprisingly, there are no gross defects in retinal patterning, synaptogenesis or neuronal survival in the absence of MG. Using this model, we assayed the functional consequences of this loss on vision using in vivo imaging and behavioral testing. Retinal circuits lacking MG respond to light stimulation with a decreased electroretinogram response. The optokinetic response is reduced but still present, however there are defects in response to changes in spatial and temporal stimulation. Measuring the activity of bipolar cells and Amacrine cells with GCaMP transgenics in vivo show a normal response to luminance levels, however have a decreased response to contrast. More specifically, as the frequency of light stimulation increases, terminals without MG stop responding at significantly lower frequencies in comparison to control. Thus, we have a novel system to test the function of neural circuits absent glial contacts.

114. Exploring the roles of Transmembrane channel-like proteins in mechanotransduction in zebrafish hair cells. ***Reo Maeda***[1,2], *Weike Mo*[1,2], *Katie Kindt*[1,2], *Timothy Erickson*[1], *Clive P. Morgan*[1], *Adam Therneau*[1], *Rachel Clemens-Grisham*[1], *Peter G. Barr-Gillespie*[1], *Teresa Nicolson*[1]. 1) Oregon Hearing Research Center and Vollum Institute, Oregon Health & Science University, Portland, OR; 2) equally contribution.
Hair cells are specialized sensory receptor cells of both the auditory and vestibular system in vertebrates. The common feature of hair cells is the hair bundle, which is the sensory organelle formed by the ordered, staircase-like structure of stereocilia. Mechanical deflection of hair bundles increases the tension of tip links that connect the stereocilia, and converts mechanical force into a neural signal. These tip links are thought to gate transduction channel. However, the identity of the transduction channels in hair cells is still unknown, although several candidate proteins have been proposed.
Here, we sought to identify proteins that interact with Protocadherin15a (Pcdh15a), which is a component of tip links. Using a membrane-based yeast two-hybrid screen, we identified an N-terminal fragment of Transmembrane channel-like 2a (Tmc2a). Tmc2a is an orthologue of mammalian TMC2. Recent studies reported that TMC1 and TMC2 are components of the mechanotransduction channel in mammalian hair cells. In zebrafish, there are one *tmc1* and two *tmc2* genes. Our yeast two-hybrid assays show that zebrafish Tmc1 and Tmc2a can interact with two isoforms of Pcdh15a, -CD1 or CD3 that have divergent cytoplasmic domains. *In situ* hybridization revealed that expression of *tmc1*, *tmc2a*, and *tmc2b* is restricted to hair cells, suggesting that these genes have specialized roles in hair cells. To investigate the roles of zebrafish Tmc2a in mechanotransduction in hair cells, we overexpressed N-terminal fragments of Tmc2a in hair cells to interfere with the interaction between Pcdh15a and Tmc2a. Upon overexpression, we detected significant decreases in mechanosensitivity. This result suggests that the interaction between Pcdh15a and Tmc2a is critical for mechanotransduction in zebrafish hair cells. Taken together with previous studies in mammals, our results support the idea that TMCs are key components of the mechanotransduction complex in hair cells.

115. In toto imaging of the developing endolymphatic duct and sac reveals cycles of tissue inflation and deflation. ***Ian A. Swinburne***, *Ebaa F. Al-Obeidi, Sean G. Megason*. Systems Biology, Harvard Medical School, Boston, MA, MA.
Disturbances of endolymph homeostasis are believed to cause Meniere's disease and Pendred Syndrome. The work of many clinical and basic researchers suggests that the endolymphatic duct and sac has an important role in endolymph homeostasis. However, understanding how the endolymphatic duct and sac form and function has been elusive because in most vertebrates they are buried within the dense temporal bone. We present the integration of imaging, genetic, and embryological approaches to describe the early development of a novel endolymphatic duct and sac physiology. The development of the endolymphatic duct and sac begins at 36 hours post fertilization and is driven by evagination of a small number of cells in the anterior-lateral portion of the dorsal otic vesicle. Tissue treadmilling repositions the endolymphatic sac to a more medial and central position in the dorsal otic vesicle. Beginning at 60 hours post fertilization the duct and sac inflate with endolymph. The inflation is dramatic as the sac transitions from being closed to holding volumes as large as 2,500 cubic microns. Inflation is followed by rapid deflation and these cycles repeat every 2-3 hours. The rate of inflation is slower than the rate of deflation. By tracking a fluorescent dye in the perilymph we discover that the collapse is preceded by the breaking of the diffusion barrier of the sac's epithelium. Inflation and collapse coincide with the dynamic behavior of cell-cell junctions that behave like a relief valve. Towards understanding how this physiology develops we characterized an endolymphatic sac deflation phenotype in an *lmx1bb* mutant reminiscent of Enlarged Vestibular Aqueduct Syndrome. In *lmx1bb* mutant embryos the sac continues to inflate without deflation and perilymph does not enter the lumen of the inflated sac. The mutant sac also lacks the specialized cell-cell junctions seen in the wild type sac. Together our results support a novel inflation and deflation physiology that initiates and cycles during ear development.

116. Sdf1-expression Reveals a Source of Perivascular-Derived Mesenchymal Stem Cells in Zebrafish. *Michelle Carter, Nardina Nash, Paul Stadem, Ashley Kramer,* ***Troy Lund***. Pediatric Blood and Marrow Transplant, University of Minnesota, Minneapolis, MN.
There is accumulating evidence that mesenchymal stem cells (MSC) have their origin as perivascular cells (PVC) in vivo, but precisely identifying them has been a challenge, as they have no single definitive marker and are rare. We have developed a fluorescent transgenic vertebrate model in which PVC can be visualized in vivo based upon sdf1 expression in the zebrafish. Prospective isolation and culture of sdf1DsRed PVC demonstrated properties consistent with MSC including expression of acta1a, cspg4, tgln, and cdh5. Cells also displayed prototypical cell surface marker expression including nnte5, thy-1, and engl (human homologs of CD73, CD90, CD150). Cultured PVCs were able to undergo mesodermal differentiation into adipogenic, osteogenic and chondrogenic lineages, were growth response to bFGF,

and displayed the ability to support hematopoietic cells. Global proteomic studies performed by 2-dimensional liquid chromatography and tandem mass spectrometry revealed a proteome with a high degree of similarity to human MSC and also revealed the discovery of novel markers (CD99, CD151 and MYOF) that were previously unknown to be expressed by hMSC. We were able to perform co-cultured experiments with cultured PVC and human endothelial cells to show an ex vivo conserved cellular interaction between both cell types. Dynamic in vivo imaging during fin regeneration showed that PVC may arise from undifferentiated mesenchyme during regeneration providing further evidence of a PVC - MSC relationship. Functionally, PVC may serve to assist in maintaining vascular integrity during regeneration as shown through vascular league studies during regeneration. This is the first model, established in zebrafish, in which MSC ancestral cells can be visualized in vivo and will allow us to better understand their function in a native environment.

117. Genetic Analysis and Characterization of the Novel Zebrafish Podocyte Mutant *zeppelin*. ***Paul T. Kroeger***[1], *Rachel Miceli*[1], *Michael McKernan*[1], *Annemarie Fox*[1], *Rachel Bounds*[1], *Jennifer Cihlar*[1], *Ignaty Leshchiner*[2], *Alan J. Davidson*[3], *Wolfram Goessling*[2], *Rebecca A. Wingert*[1]. 1) University of Notre Dame, Notre Dame, IN; 2) Harvard Medical School and Brigham and Women's Hospital, Boston, MA; 3) University of Auckland, Auckland, NZ.
The zebrafish pronephros is highly conserved with higher vertebrates, including mammals, thus making it an excellent model to study kidney formation. The nephron, or functional unit of the kidney, modifies the blood to excrete metabolic waste using a blood filter with specialized epithelial cells known as podocytes. Knowledge about podocyte development is highly relevant to the treatment and prevention of kidney disease, as podocyte injury leads to progressive scarring and nephron atrophy culminating with end stage renal failure, however the pathways that specify the podocyte lineage remain poorly understood. Through an edema based F3 forward genetic screen, we isolated *zeppelin* (*zep*), which displays edema at 5 days post fertilization and forms severely reduced podocytes as assayed by *in situ* hybridization with markers such as *wt1a, wt1b, lhx1a*, and *nephrin. zep* mutant embryos are unaffected by retinoic acid (RA) treatment, indicating that RA acts upstream or in an unrelated pathway with that of *zep*. Interestingly, the interrenal gland of *zep* mutants is increased in size. Preliminary cell death and proliferation assays in *zep* mutants did not show any alterations from wildtypes, suggesting the possibility of a cell fate switch between the podocyte and interrenal lineages. To determine the genetic lesion responsible for *zep*, we utilized a combinatorial strategy of whole genome sequencing (WGS) and meiotic mapping. These techniques narrowed the region to a small interval on chromosome 15, and candidate genes were knocked down with morpholinos (MO). Three independent MOs designed against *breast cancer 2, early onset* (*brca2*) phenocopied *zep*, recapitulating both the unique late edema phenotype, as well as the vast reduction of podocytes. This suggests for the first time that *brca2* is essential for renal development. Taken together, these findings provide novel insights into the genetic regulatory networks that control podocyte formation in the vertebrate kidney.

118. Transcriptome profiling of adult zebrafish centroacinar cells reveals their similarity to larval endocrine pancreas progenitors. ***R. L. Beer***[1], *G. Wang*[1], *F. Delaspre*[1], *W. Huang*[1], *S. Gee*[2], *M. Rovira*[3], *M. Bagnat*[4], *S. Wheelan*[1], *M. J. Parsons*[1]. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 3) Genomic Regulation of Pancreatic Beta-Cells Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain; 4) Department of Cell Biology, Duke University Medical Center, Durham, NC.
A deeper understanding of the progenitor populations that give rise to b cells during embryonic and adult development can inform therapies for treatment of type 1 diabetes. Centroacinar cells (CACs) are a population of Notch-responsive cells located in a terminal duct position in the vertebrate pancreas. In the larval zebrafish pancreas, using genetic lineage tracing we have previously shown that pancreatic Notch-responsive cells (PNCs) are progenitor cells that give rise to adult endocrine cells, ductal cells, and CACs. Here, we demonstrate that CACs are a progenitor population that contributes to b-cell regeneration following b-cell specific ablation in the zebrafish pancreas. We next sought to characterize this adult endocrine progenitor population using RNA-seq. To do so we purified CACs by fluorescence activated cell sorting of dissociated pancreata from transgenic Tg(*tp1:eGFP*) adults, which express GFP in Notch-responsive cells. During sorting, Notch-responsive endothelial cells were removed by 1) a CD105 antibody or 2) a Tg(*fli1:RFP*) transgenic line. Comparing transcripts differentially regulated (logFC>3.0, $p<0.05$) in CACs (GFP+/RFP-) vs all other pancreas cells (GFP-/RFP-) we identified 236 genes that were up-regulated in CACs. Functional annotation clustering of gene ontology terms for these 236 genes revealed enrichment for embryonic patterning, cell-cell contacts, epithelial development, and cell motility terms. We next validated the expression of four of the most highly up-regulated genes: *cftr, clcn1b, her15*, and *nkx6.1*. Interestingly, we observed expression of these genes in both PNCs and CACs. Thus we conclude that PNCs are a larval population of CACs. Additionally, we have now developed a useful set of markers to further characterize the role of CACs during b-cell development and regeneration.

119. Pseudophosphatase dusp27 is a novel sarcomere protein and required for the development of the skeletal muscle contractile apparatus. ***Eric Horstick***[1], *Kandice Fero*[1], *Sadie Bergeron*[1], *Hiba Codore*[1], *Grace Li*[1], *Fumihito Ono*[2], *James Dowling*[3], *Harold Burgess*[1]. 1) Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD 20892, USA; 2) Section on Model Synaptic Systems, Laboratory of Molecular Physiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852, USA; 3) Division of Neurology, Hospital for Sick Children, Toronto, ON M5G 1X8, Canada.
Muscle contraction is dependent on the specialized and highly organized architecture of sacromeres, the contractile basis of myofibes. Despite significant understanding of the structural and functional role of individual proteins in sacromeric contraction, the developmental mechanism governing assembly is poorly understood. We identified a zebrafish motor mutant with severely reduced embryonic and larval motility caused by the integration of an enhancer trap transgene into dual specificity phosphatase 27 (dusp27). Intriguingly, dusp27 lacks a conserved catalytic cysteine residue and is therefore very likely not an active phosphatase, and represents a unique subtype within the DUSP protein family. Expression analysis of dusp27 revealed strong expression in skeletal and cardiac muscle. Further characterization of muscle showed that the loss of dusp27 did not impair myofiber formation, maturation, or somitogenesis, yet severely impaired assembly of the contractile apparatus. In dusp27 mutant fast twitch muscle, none of the tested structural components were localized normally and only

rare triadic structures could be observed by electron microscopy. However, slow twitch fibers maintained grossly normal Z-lines, suggesting variable requirements for dusp27 during fiber type myofibrillogenesis. Expression of EGFP-dusp27 in mutant myofibers restored sacromeric protein localization in fast twitch fibers and demonstrated that dusp27 localizes in a pattern consistent with sarcomere proteins. Interestingly, the localization of Dusp27 to the sarcomere suggests a role in sarcomere contraction or maintenance, independent of initial formation. These data are the first to describe a functional role for dusp27.

120. Elucidating the Mechanisms of Therapeutic Augmentation of Kidney Repair After Acute Kidney Injury. ***Lauren Brilli***[1], *Takuto Chiba*[3], *Nataliya Skrypnyk*[4], *Lee Mcdermott*[2], *Mark De Caestecker*[3,4], *Neil Hukriede*[1]. 1) Developmental Biology, University of Pittsburgh, Pittsburgh, PA; 2) Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA; 3) Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN; 4) Medicine, Vanderbilt University Medical Center, Nashville, TN.
Acute kidney injury (AKI) is a serious disorder for which there is no targeted clinical treatment. A promising approach to improve treatment options lies in developing novel post-AKI therapies that enhance innate renal regenerative processes. Our lab has used zebrafish to identify small molecules that enhance renal tubular epithelial cell (RTEC) regeneration after AKI. Using this approach we identified methyl-4-phenylthiobutanoate (m4PTB), an HDAC inhibitor that promotes renal progenitor expansion in zebrafish embryos and accelerates recovery after AKI in zebrafish and mice. The ability of m4PTB to expand the renal progenitor cell population in zebrafish embryos depends on intact retinoic acid (RA) signaling, but the mechanisms underlying how m4PTB accelerates recovery after AKI are unknown. To elucidate these mechanisms, we utilize a nephrotoxic model of gentamicin-induced AKI in zebrafish larvae that demonstrates the same hallmarks of renal injury and regeneration post-AKI as the mammalian kidney. Tg(12XRARE:GFP) transgenic larvae are used to assess RA signaling, and heat shock is used to induce expression of a dominant negative RA receptor in Tg(hsp70:dnRARa) larvae. Live imaging of Tg(12XRARE:GFP) larvae post-AKI shows that m4PTB stimulates RA signaling in the larval zebrafish kidney RTECs. m4PTB also increases expression of the RA pathway components Aldh1a2 and Ret by qRT-PCR in a mouse model of ischemia reperfusion AKI. Finally, we show that reducing RA signaling by heat shock in Tg(hsp70:dnRARa) larvae abrogates m4PTB-stimulated RTEC proliferation and impairs larval survival post AKI. These studies indicate that m4PTB activates RA signaling and that m4PTB-dependent renal regeneration requires intact RA signaling post-AKI. This work suggests that m4PTB action is mediated by activating RA-dependent regenerative responses after AKI.

121. Phosphoinositide recycling and angiogenesis. *Amber M. Stratman, Coinstantinos M. Mikelis, Weijun Pan, Van N. Pham, Tina M. Kilts, George E. Davis, J. Solivo Gutkind,* ***Brant M. Weinstein***. 1) Program in Genomics of Differentiation, NICHD, NIH, Bethesda, MD; 2) Oral and Pharyngeal Cancer Branch, NIDCR, NIH, Bethesda, MD.
Anti-angiogenic therapies have been regarded as one of the most promising new approaches for combating cancer, but they have yet to fulfill this promise. In large part, this is because of the ability of tumors to evade or overcome these therapies by up-regulating pro-angiogenic cytokines. We report a new approach to inhibit angiogenesis - targeting recycling of the rate-limiting substrate used for transduction of VEGF-dependent angiogenic signaling. Beginning with zebrafish mutants identified in a genetic screen and using zebrafish, endothelial cell culture, and mouse tumor models, we show that this new approach has the potential to result in a uniquely effective inhibition of tumor angiogenesis, since increased VEGF stimulation, rather than overcoming the inhibition, only results in faster consumption of the limiting substrate and more rapid and complete inhibition of angiogenesis.

122. Mutations in *ext* genes reveal that Heparan Sulfate Proteoglycans regulate Wnt/b-catenin and Fgf signaling domains in the lateral line primordium. ***Marina Venero Galanternik***[1,2], *Kenneth Kramer*[3], *Tatjana Piotrowski*[1,2]. 1) Department of Neurobiology and Anatomy, University of Utah, UT; 2) Stowers Institute for Medical Research, Kansas City, MO; 3) Department of Biomedical Sciences, Creighton University, NE.
The posterior lateral line (pLL) sensory system develops from a migrating placode called the pLL primordium (pLLp) and represents a powerful model to study vertebrate organ development. Proper activation of the Wnt/b-catenin and Fgf signaling pathways is vital for correct pLL development. Heparan Sulfate Proteoglycans (HSPGs) are extracellular matrix glycoproteins that bind signaling molecules through their modified sugar chains and are critical for the precise activation of several signaling pathways such as Wnt, Fgf, Hh and BMP. HSPGs also could affect the diffusion of these signaling molecules. In vertebrates, the in vivo function of HSPGs is still not well understood. We identified four HSPGs that are expressed in restricted domains of the pLLp and are under the control of Wnt/b-catenin and Fgf signaling. Pharmacological disruption of HSPGs and analysis of *ext3l/ext2* mutants, that lack functional HSPG chains, revealed that HSPGs are crucial for the proper localized activation of Wnt/b-catenin and Fgf signaling in the pLLp. In the absence of functional HSPGs, Fgf signal transduction fails to occur, even in the presence of abundant Fgf ligands; Wnt/b-catenin signaling expands along the primordium and the chemokine receptor *cxcr7b* is downregulated triggering pLLp stalling. We rescued Fgf signal transduction by constitutively activating *fgfr1* in HSPG-depleted embryos, demonstrating that HSPGs are necessary for Fgf receptor function. Additionally, induction of the Fgf target and Wnt inhibitor Dkk1b restored the boundary between the Wnt/b-catenin and Fgf domains. These results suggest that HSPGs do not affect Fgf or Wnt ligand diffusion in the pLLp. Instead, the loss of Fgf signaling causes the loss of *dkk1b* leading to the expansion of the Wnt/b-catenin domain. Our studies lead to a better understanding of the mechanisms underlying Wnt/b-catenin and Fgf pathway activation during development, as well as provide important insights into HSPG-dependent signaling.

123. The Iroquois transcription factors Irx7 and Irx5a promote the zebrafish hyoid joint by arresting chondrocytes at an early state of maturation. ***Amjad Askary****, Xinjun He, Lindsey Mork, Shinsuke Ohba, Andrew P. McMahon, Gage Crump.* Broad CIRM Center, Keck School of Medicine, University of Southern California, Los Angeles, CA.
Joints provide essential mobility to the vertebrate skeleton, and arthritic degeneration of joints is the leading cause of disability in humans. An early event in joint development is the creation of an “interzone” between two prospective skeletal elements. Interzone cells are

maintained as fibroblasts and/or articular chondrocytes while flanking cells mature into hypertrophic chondrocytes. Whereas a number of studies highlight the importance of BMPs and Gdf5/6 in balancing hypertophic and articular cartilage, respectively, much less is known about their downstream targets during joint development. Here we show that two Iroquois transcription factors, Irx7 and Irx5a, are negative targets of BMPs that arrest the further differentiation of early chondrocytes in the hyoid joint of zebrafish. Iroquois transcription factors are expressed in joints of amniote limbs, and a large deletion encompassing Irx3, Irx5, and Irx6 is associated with digit fusions in mice. Interestingly, an additional Irx gene - irx7 - exists only in fishes, where it is expressed exclusively within the fish-specific hyoid joint. We find that, like the jaw joint marker nkx3.2, both irx7 and irx5a become restricted to the hyoid joint by the combined actions of Endothelin1 signaling and Hand2, Barx1, and Hox transcription factors. We also generated irx7 and irx5a mutants, using TALEN technology, and find that these display specific losses of the hyoid joint and adjacent symplectic cartilage. Conversely, mosaic Irx7 misexpression inhibits chondrocyte differentiation in a cell-autonomous manner. Irx7 and Irx5a appear to arrest chondrocyte differentiation downstream of SoxE factors by directly binding to two classes of Sox9-bound Col2a1 enhancers. While type I enhancers are excluded from joints and repressed by Irx7/5a, type II enhancers are preferentially expressed in joints and activated by Irx7/5a. This opposing regulation suggests that Irx genes promote joints by specifying a precise low level of col2a1a in early-chondrocyte-like interzone cells.

124. Cellular asymmetry in angiogenesis revealed by live imaging in zebrafish. ***Donna J. Page***[1], *Didier Y. Stainier*[2], *Shane P. Herbert*[1]. 1) Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; 2) Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.
During angiogenesis, sprouting endothelial cells are hierarchically organized into specialized leading “tip” cells and trailing “stalk” cells. Consequently, coordinated maintenance of the tip-stalk cell hierarchy is critical for normal blood vessel formation. We have identified and are functionally interrogating several genes that play key roles in maintaining the tip-stalk hierarchy. However, the mechanisms by which tip/stalk cell identity and behavior are inherited or re-established following cell division are currently unclear. Using multiplexed real-time imaging approaches in zebrafish embryos we provide the first evidence for cellular asymmetry following mitosis in angiogenesis. Extensive quantitative analysis of tip/stalk cell behavior *in vivo* reveals that tip cell identity is asymmetrically acquired following tip cell division to generate daughter cells with distinct stereotyped behaviors. Similarly, in the absence of Dll4-Notch signaling, stalk cells not only obtain ectopic tip cell identity but also acquire asymmetric divisions. Importantly, we identify a Notch-regulated tip cell-restricted tetraspanin-like gene, *tm4sf1*, which functions to induce tip cell motility and behavior in one daughter cell following tip cell division. This work and ongoing studies investigating the functional interaction of Tm4sf1 with Vegf signaling indicates a previously unknown role for cellular asymmetry in angiogenesis.

125. Motor exit point (MEP) glia: Novel CNS-derived glia that segregate myelinating glia at the MEP. ***Sarah Kucenas***, *Cody Smith, Angela Morris, Taylor Welsh.* Department of Biology, University of Virginia, Charlottesville, VA.
Rapid conduction of action potentials along motor axons requires that oligodendrocytes and Schwann cells myelinate distinct central and peripheral nervous system (CNS and PNS) domains along the same axon. Despite the importance of this glial arrangement for nervous system function, the mechanisms that establish and maintain selective glial segregation at the motor exit point (MEP) are unknown. Using in vivo time-lapse imaging in zebrafish, we identified a novel, CNS-derived population of glia we call MEP glia that are essential for restricting oligodendrocytes to the spinal cord at the MEP. Ablation of MEP glia results in the absence of myelinating glia along spinal motor root axons in the periphery and an immediate breach of the MEP by oligodendrocyte progenitor cells (OPC). Together, our results identify a novel population of CNS-derived peripheral glia located at the MEP that establish and maintain segregation of myelinating glia at the MEP.

126. 4-hydroxycoumarin Induced Developmental Deformities in Zebrafish (Danio rerio). ***Narshimamurthy Anegundi***, *Pancharatna A Katti*. Dept of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India.
Teratogenic effects 4-hydroxycoumarin (a derivative of coumarin) were studied on the development of zebrafish (Danio rerio) by exposing the developing embryos (n = 20) to graded (1.0, 2.0, 3,0, 4.0 and 5.0 mM) concentrations of the chemical from 6 hpf onwards. After hatching at 72 hpf, larvae were observed for morphological abnormalities and developmental anomalies if any, under microscope and photographed. No abnormalities or mortality were observed in 1.0 - 3.0 mM concentrations and larvae developed from embryos of these groups were comparable to controls while, those larvae developed from embryos exposed to 4.0 and 5.0 mM 4 hydroxycoumarin, 50% of larvae died, while the other 50% had one or the other anomalies such as, tail bending, edema around heart, deformities in cephalic and trunk region. Angiogenesis also seem to be affected and blood flow in intra segmental vessels (ISV) was hampered.

127. Optical Control of Tumor Initiation from Single Cells in Zebrafish. ***Zhiping Feng***[1], *Tal Markus*[2], *Sophie Vriz*[3], *Michel Volovitch*[3,4], *Ludovic Jullien*[5], *Shuo Lin*[6], *Shimon Weiss*[1,7], *David Bensimon*[2,7]. 1) Department of Molecular, Cellular and Integrative Physiology, University of California Los Angeles, Los Angeles, CA 90095, USA; 2) LPS, UMR 8550 CNRS, 24 rue Lhomond and IBENS, 46 rue d’Ulm, Ecole Normale Superieure, ' Paris 75005, France; 3) College de France, Center for Interdisciplinary Research in Biology, (CIRB), CNRS UMR 7241-INSERM ` U1050, 11 place Marcelin Berthelot, and Univ Paris Diderot, Sorbonne Paris Cite, Paris, F-75005, France; 4) Department of Biology,LPS, École normale supérieure, Paris, France; 5) Department of Chemistry, Ecole Normale Superieure, UMR CNRS-ENS-UPMC 8640, 24 rue Lhomond, ' 75005 Paris, France; 6) Department of Molecular, Cell & Developmental Biology, University of California, Los Angeles, CA 90095, USA; 7) Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA 90095, USA.
Central events in cancer development are believed to take place as rare occurrences at the level of individual cells or small groups of cells. However, study of cancer evolution from single transformed cells is extremely challenging and has yet not been well characterized. Recently, we developed a technology that allows for the control of protein activity and gene expression in single cells through light

activation. In this work, we utilize this method to activate in individual cells of a zebrafish a typical oncogene, K-RasG12V and investigate effects of these changes on tumorigenesis with both wild type and p53 mutant zebrafish. We have demonstrated the success of spatiotemporally controlling oncogene expression in live zebrafish. Furthermore, we investigated different tumorigenic phenotypes by transiently or permanently activating K-Ras at varied developmental stages. we believe our study could open up a completely new basis for understanding cancer growth and eventually testing anti-cancer drugs.

128. Deciphering the genetic interactions responsible for p53 protein stability. ***Amber Guidry***, *Holly Thomas, John Parant*. University of Alabama at Birmingham , Birmingham, AL.
The regulation of the tumor suppressor gene p53 is critical in maintaining normal cellular growth and preventing tumorigenesis. Approximately half of all human cancers have a mutation in p53; the remaining 50% of cancers that do not contain mutations directly in the p53 gene usually have genetic alterations that disrupt the regulation of the p53 pathway resulting in tumor formation. For example, the dysregulation of p53 stability through the overexpression of Mdm2 or Mdm4, which are both negative regulators that target p53 for degradation, has been found in over twenty percent of all human cancers. Although knockouts of these p53 regulators have been generated in mice, detailed embryonic analysis has proven to be difficult due to their early embryonic lethality. Our lab has previously shown that p53 mutant zebrafish develop early onset and highly penetrant sarcomas; the p53 protein is stabilized following DNA damage; and p53 activation induces a conserved set of p53 target genes. These results demonstrate that zebrafish, which undergo external fertilization and transparent embryogenesis, is an excellent model to study the highly conserved p53 pathway. To generate zebrafish with knockout mutations in critical p53 regulators, including Mdm2, Mdm4, and Hausp, we have utilized two of the latest genomic-editing technologies: Transcription Activator-Like Effector Nucleases (TALENs) and the CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins) system. We are currently performing detailed embryonic characterization of several mutant zebrafish lines; I will discuss our progress on analyzing each of these p53 regulatory models. As anticipated based on previous studies, we found that our homozygous Mdm2 mutants are early embryonic lethal and exhibit an increase in cellular apoptosis. To determine if this phenotype is dependent on p53, we utilized our previously established p53 morpholino to knockdown p53 expression in Mdm2 homozygous embryos. The knockdown of p53 in the homozygous Mdm2 mutants restored proper development throughout the embryos. Our results indicate that Mdm2 plays a critical role in zebrafish development through its regulation of p53 protein stability.

129. Genetic Approaches To Define Novel Therapeutic Targets In Germ Cell Tumors. ***Vinita Hajeri***[1], *Maura Mcgrail*[2], *Jeffrey Essner*[2], *Bruce Draper*[3]. 1) UT Southwestern Medical Center, Dallas, TX; 2) Iowa State University, Ames, IA; 3) UC-Davis, Davis, CA.
Germ Cell Tumors (GCTs) are the most common malignancy of young men aged 15-40 years and a frequent childhood cancer. For 30 years, GCTs are treated with cisplatin-based chemotherapy, which while effective produces detrimental adverse effects including risk of secondary cancers. The molecular mechanisms driving GCT are unclear, limiting the development of alternative therapies. We previously showed that disruption of the BMP pathway in zebrafish causes GCTs similar to humans (Neumann et al, 2011). Capitalizing on zebrafish as a GCT model, we are conducting a forward genetic screen using a Sleeping Beauty transposon-based insertional mutagenesis strategy to uncover novel genes involved in GCT pathogenesis. Cells expressing SB100X transposase randomly insert the T2/OncZ transposon creating loss-of or gain-of function mutations, mimicking tumorigenesis in sporadic cancers. To establish our screen, we created a transgenic line of fish expressing germline specific SB100X transposase. We hypothesize that mobilization of transposon concatemers in the zebrafish germline will cause random somatic mutations leading to GCT development. Double transgenic *T2/OncZ;SB100X* fish are being raised and monitored for GCT tumors. Tumor DNA from the GCT harboring fish is being analyzed by next-generation sequencing to identify candidate genes involved in GCT tumorigenesis. Secondly, we are investigating the role of deregulated Fibroblast Growth factor (FGF) signaling in germ cells. Studies on GCT samples have highlighted the role of FGF proteins including FGF8 and Sprouty4, but *in vivo* animal models to determine the pathological mechanisms by which SPRY4 and FGF proteins promote tumorigenesis are unknown. SPRY4 is involved in germ cell biology through its interaction with the KITLG/KIT and Ras/MAPK signaling pathways. We are generating transgenic lines of zebrafish overexpressing wildtype, dominant negative SPRY4 (dnSPRY4) and FGF8 forms in germ cells. Together, these genetic approaches will provide mechanistic insights into pathogenic drivers of GCT, serving as a platform to evaluate novel therapeutic approaches.

130. In vivo Analysis of the NF1 Tumor Suppressor in Neuroblastoma Pathogenesis. ***Shuning He***[1], *Dong Hyuk Ki*[1], *Shizhen Zhu*[1], *Eric D. de Groh*[2], *Jonathan A. Epstein*[2], *A. Thomas Look*[1]. 1) Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 2) Department of Cell and Developmental Biology, Penn Cardiovascular Institute, and the Institute for Regenerative Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA.
Neuroblastoma is an embryonic malignancy of the peripheral sympathetic nervous system (PSNS) and accounts for 8-10% of all childhood malignancies and 15% of all cancer deaths in children. Several genes have been implicated that represent putative oncogenic drivers or tumor suppressors in high-risk cases of neuroblastoma. However, how these genetic alterations (such as MYCN amplification, ALK activation and loss of NF1) elicit molecular and cellular changes leading to neuroblastoma remain unknown. The neurofibromatosis type 1 gene (NF1) is one of the most prevalent tumor suppressors in human high-risk neuroblastoma. The large NF1 protein contains a GTPase-activating protein-related domain (GRD) and NF1-loss results in aberrant activation of Ras signaling and predisposes NF1 patients to a variety of cancers. We have identified two separate zebrafish nf1 genes and used a zinc-finger nuclease strategy to generate multiple loss of function nf1 mutant zebrafish lines. Accelerated development of sympathetic ganglia and expansion of superior cervical ganglion was observed in nf1 mutant zebrafish starting at 3 days post-fertilization, as well as ectopic cells expressing the sympathetic neuronal lineage marker dopamine beta-hydroxylase, suggesting aberrant PSNS cell migration. To study the role of NF1 in neuroblastoma tumorigenesis, we intercrossed nf1 mutant zebrafish with a transgenic zebrafish neuroblastoma model that overexpresses human MYCN in the PSNS. Fish lacking two alleles of nf1a and one allele of nf1b exhibited a greatly accelerated onset of neuroblastoma induced by MYCN

overexpression, with a penetrance of nearly one hundred percent by 5 weeks of age. The nf1a-/-, b+/- fish developed much larger tumors than wild-type fish overexpressing MYCN, suggesting that these tumor cells have a much faster proliferation rate in the nf1 mutant background.

131. Rapid in vivo transformation of hepatic cells upon activation of oncogenes in Tet-On Inducible Transgenic Zebrafish. ***X. Huang***[1], *Y. Zhao*[1], *J. Spitsbergen*[2], *Z. Gong*[1]. 1) Department of Biological Sciences, National University of Singapore, Singapore; 2) Department of Microbiology and Marine and Freshwater Biomedical Sciences Center, Oregon State University, Corvallis, OR, USA 97331.
Hepatocellular carcinoma (HCC), ranked the fourth leading cause of tumor mortality worldwide, is also the most common type of primary cancers in the liver. Although major discoveries have been made in cellular and molecular mechanisms underlying liver carcinogenesis, there is still a lack of good animal models to capture the key events during liver tumor initiation. Our laboratory has successfully generated several Tet-On oncogene transgenic zebrafish with inducible liver tumors. In this study, juvenile single (Myc or xmrk) and double Myc/xmrk transgenic zebrafish were induced with doxycycline and we found that hepatic cells transformed to hyperplastic and HCC phenotypes in xmrk and Myc/xmrk double transgenic fish, with 100% penetrance and homogeneity, within one week. A decrease of glycogen deposit in transforming hepatocytes was observed at the early stage, while there was an increase of nuclear:cytoplasic ratio, indicating an augmentation of cell malignancy. Although fibrosis was not detected, a sign of initial increment of lipid content in the hepatic cells was observed in all three transgenic tumor models. Interestingly, other than active cell proliferation, extensive apoptotic signals were also detected at the early tumor stage, with xmrk tumor exhibiting the most number of apoptotic cells. By crossing our tumor lines with Tg(fli:EGFP) fish, an increase of blood vessel density was observed in both Myc and Myc/xmrk livers. The role of angiogenesis in tumor initiation was further confirmed by treating fish with angiogenesis inhibitors upon doxycycline induction, and a significant reduction of liver size was recorded. In addition, inflammation also occurred during tumorigenesis, indicated by an influx of neutrophils into liver. Altogether, these results suggest immediate response of immune cells, increased angiogenesis, increased cellular metabolism upon the induction of an oncogene and these may be the common response during tumor initiation in vivo.

132. Number not programmed.

133. Transcriptomic profile of tumor associated neutrophils reveals active migration ability and suppression of immune. ***Xiaojing Huo***[1], *Chuan Yan*[2], *Zhen Li*[3], *Sinnakaruppan Mathavan*[3], *Zhiyuan Gong*[1,2]. 1) DBS, NUS, Singapore; 2) NGS, Singapore; 3) GIS, Singapore.
Recently we observed a rapid recruitment of neutrophils to oncogenic livers in our *kras*^{V12} transgenic zebrafish model with inducible hepatocellular carcinoma and an obvious promoting role of neutrophils in hepatocarcinogenes. However, little is known about the molecular mechanisms of the promotional role of neutrophils. Thus, an RNA-Seq approach was used to compare transcriptomic profiles of tumor associated neutrophils (TANs) against naive neutrophils (NNs). Two striking features were revealed from the comparison: active cell migration and down-regulation of immune responses in TANs. Cell migration, fibrin clotting cascade and intrinsic pathway were enriched in TANs, suggesting that the signaling of neutrophil recruitment to liver tumor might be similar to that in wound healing. Consistent with this, Tgf-b a main inducer of neutrophil chemotaxis in wound healing, was predicted by IPA (Ingenity Pathway Analysis) as one of the top upstream regulators in TANs. This was further validated by antibody depletion of Tgf-b in 5 dpf larvae and we observed decreased migration of neutrophils and delayed liver tumor progression, indicating a Tgf-b-dependent mechanism for TAN recruitment. Furthermore, the pro-tumor effect in TANs might also be due to the suppression of anti-tumor immune pathways (e.g. TNF-a pathway, IFN-g pathways) and the down-regulation of activating transcription factor 2 pathway, which regulates expression of JUN, IFN-g, SOCS3, as revealed from the RNA-Seq data. To gain a comprehensive view of the host-tumor interaction as well as the role of Tgf-b in tumor-promoting inflammation, transcriptome of oncogenic hepatocytes and Tgf-b-depleted neutrophils are being analyzed. By elaborating the molecular mechanism of neutraphil recruitment and pro-tumor effect, it is possible to identify and validate new immune-diagnostic markers for liver tumor from the cluster of cell migration and to predict drug targets for adjunctive therapy of liver tumor based on the gene sets of immune responses and tumor-promoting inflammation.

134. The Histone Demethylase *kdm6bb* is Required for *nkx2.5* Expression and Second Heart Field Development. ***Alexander Akerberg***[1,2], *Scott Stewart*[2], *Kryn Stankunas*[1,2]. 1) Institute of Molecular Biology, University of Oregon, Eugene, OR; 2) Department of Biology, University of Oregon, Eugene, OR.
As with all vertebrates, the zebrafish heart is largely comprised of tissues derived from two progenitor populations referred to as the first and second heart fields. Cells of the first heart field (FHF) migrate to the midline to establish the primitive heart tube. Subsequently, cells of the second heart field (SHF) accrete to the poles of the heart tube to form, most notably, the anterior ventricle and outflow tract. Cardiogenic transcription factors (TFs) such as *nkx2.5*, *tbx5*, and *gata4* cooperate to establish, maintain, and direct the differentiation of both FHF and SHF cells. These TFs function within chromatin landscapes to promote cardiac gene expression programs. However, the effect of local chromatin states on cardiogenic TF activity is poorly understood. Recent evidence suggests that dynamic histone H3 lysine-27 tri-methylation (H3K27me3), a repressive mark established by the Polycomb Repressive Complex 2 (PRC2), accompanies gene expression changes during cardiogenesis. We examined the in vivo roles of H3K27me3 regulators during zebrafish heart development and found that one H3K27me3-specific histone demethylase, *kdm6bb*, was highly expressed throughout this process. Morpholino-mediated knockdown of *kdm6bb* produced embryos with a smaller ventricle, an absent outflow tract, and great artery patterning defects. The diminished ventricle and outflow tract phenotypes suggest that *kdm6bb* is essential for proper SHF development. By an RNA in situ hybridization screen, we found that *kdm6bb* morphants retained normal expression of most cardiac progenitor genes associated with the SHF. However, they showed a striking decrease in levels of *nkx2.5* within the anterior lateral plate mesoderm. We propose that Kdm6bb has a specific role in initiating *nkx2.5* expression by removing repressive H3K27me3 marks established earlier in development. To further

test this model, we are using novel zebrafish mutants to define the roles of PRC2 in heart development. Together, our results suggest that epigenetic control of H3K27me3 is a key but surprisingly specific component of cardiogenic networks.

135. Udu/Gon4l potentially regulates cardiac cell fate through histone interactions. ***Terin E Budine**, Atsushi Sawada, Margot Williams, Lilianna Solnica-Krezel.* Washington University in Saint Louis, Saint Louis, MO. 660 S. Euclid Saint Louis, MO 63110.
Congenital heart defects are among the most common birth defects in humans, and a more complete knowledge of cardiac development is critical to our understanding of the origins of these developmental defects. Cardiac development is a complex process that requires tight regulation of many signaling pathways including Wnts, Bmps and Fgfs to specify cardiac progenitor cells(CPCs) and move the CPCs into their correct orientation to form a heart. Through recent publications, it is becoming increasingly clear that histone modifications also play a major role in regulating cardiac specification. However, the mechanisms of histone regulation of cardiac specific genes are not well understood. In a forward genetic screen for PCP pathway interactors, we identified an allele of zebrafish mutant *ugly duckling* (*udu*^{vu66}), which encodes a chromatin factor. Removing both the maternal and zygotic expression of udu (MZ*udu*) results in the embryos failing to specify cardiac progenitor cells. The mouse homolog of Udu, Gon4l, has been found to complex during mouse hematopoiesis with the epigenetic modulator Yy1, which binds the regulatory regions of *Nkx2.5* during cardiac specification, suggesting a role for it in cardiogenesis. Through qRT-PCR and in situ hybridization we found that the expression of *nkx2.5* is reduced while expression of other cardiac markers like *gata4* and *nkx2.7* is up-regulated in MZ*udu* embryos. These data taken together with Western blots and microarray data showing mis-regulation of several histone deactelyases in MZ*udu* embryos suggests that Udu epigenetically regulates the expression of genes that pattern lateral plate mesoderm and specify cardiac cell fate. Our current studies test the hypothesis that Udu complexes with Yy1 and other proteins to bind the regulatory regions of lateral plate mesoderm and cardiac genes and modulate their expressions through histone modifications.

136. *Nkx* Genes Regulate Cardiomyocyte Differentiation at the Arterial Pole and Pattern the Venous Pole Through Isl1 Repression. ***Sophie Colombo**, Kimara Targoff.* Division of Cardiology, Department of Pediatrics, College of Physicians And Surgeons, Columbia University, New York, NY, 10032.
In higher vertebrates, *Nkx2-5* is expressed in both the first (FHF) and second (SHF) heart fields, two cardiac progenitor populations characterized by sequential phases of differentiation. While we previously revealed essential roles of *nkx2.5* and *nkx2.7*, two *Nkx* genes expressed in zebrafish cardiomyocytes (CMs), in maintaining ventricular identity of the FHF, their function in SHF development remains elusive. Here, we show that *nkx* genes regulate CM differentiation from the SHF at both poles of the heart, favoring differentiation at the arterial pole while restricting differentiation at the venous pole. *nkx2.5*$^{-/-}$*;nkx2.7*$^{-/-}$ embryos display a loss of CMs at the arterial pole, whereas a gain in CM number is observed at the venous pole. Furthermore, decreased *ltbp*3 and *mef2cb* expression illustrates a CM differentiation defect in the SHF progenitors at the arterial pole, yet the endocardium forms normally and development of smooth muscle cells of the bulbus arteriosus is mildly impaired. In contrast, venous pole development is more dramatically affected following the loss of *nkx* gene function. Intriguingly, in *nkx2.5*$^{-/-}$*;nkx2.7*$^{-/-}$ embryos, unrestricted expression patterns of sinoatrial node (SAN) genes, *bmp4*, *tbx2b* and *hcn4*, indicate severe abnormalities in the patterning of this venous pole derivative. Similarly, expression of the SHF marker Isl1 is expanded throughout the *nkx2.5*$^{-/-}$*;nkx2.7*$^{-/-}$ heart. Our data indicate that *nkx* genes restrict *isl1* expression to the venous pole during heart tube formation by repression in the FHF-derived myocardium, and overexpression of *nkx2.5* is sufficient to inhibit *isl1*. Moreover, we show that *nkx* genes regulate *bmp4* and *tbx2b* through expression of *isl1* specifically at the venous pole. Together, these findings suggest a pivotal function of *nkx* genes in SHF CM differentiation and venous pole patterning and highlight their essential role in preventing FHF-derived myocardium from adopting SHF characteristics. Ultimately, our results have potential to shed light on the origins of conotruncal and atrial malformations in patients carrying *NKX2-5* mutations.

137. Elucidating the endocardial function of the Heart of glass transmembrane protein. ***Stefan Donat**[1], Cécile Otten[1], Marc Renz[2], Marta Lourenco[1], Salim Abdelilah-Seyfried[1,2].* 1) Zebrafish Cardiovascular Developmental Genetics, Max Delbrück Center for Molecular Medicine, Berlin, Germany; 2) Zebrafish Cardiovascular Developmental Genetics, Hannover Medical School, Hannover, Germany.
Familial cerebral cavernous malformations (CCM) are low-blood-flow vascular lesions caused by a loss of heterozygosity at the *Krev interaction trapped protein 1* (*KRIT1*)/*CCM1*, *CCM2*, or *CCM3* loci. In mice and zebrafish, the loss of endothelium-specific proteins Ccm1, Ccm2, or of their associated transmembrane protein Heart of glass (Heg) also results in ballooned and thin-walled hearts that lack cardiac cushions. We also observed massively increased endocardial cell numbers in *heg* mutant hearts. These cardiac phenotypes point at an important morphogenetic role of Ccm proteins within the endocardium, which is a specialized region of the endothelium within the heart that contributes to cardiac cushions. To elucidate the role of endocardial signaling for cardiac morphogenesis, we focused on Heg with its two extracellular epidermal growth factor-like repeats and its short NPXY/F motif-containing cytoplasmic tail.
We initiated a structure/function analysis of Heg by generating two deletion constructs, one lacking the extracellular domain and another consisting only of the intracellular tail of *heg*, which was shown to interact with Krit1. Surprisingly, overexpression of only the intracellular tail of Heg rescued the *heg* mutant cardiac phenotypes. Hence, the membrane association of Krit1 is apparently dispensable for function. This finding indicates the importance of interactions between Heg and Krit1 and raises the question of how Krit1 localizes at adherens junctions or integrin complexes. To further elucidate the mode of interaction between Heg-Krit1 and endocardial junctional complexes, we are now analyzing tissue- and temporal-specific transgenic zebrafish that express Heg and Krit1 fusion or deletion constructs.

138. Determining the role of *PTPN12* in congenital heart disease and vascular development. ***Elizabeth A. Duffy***[1], *Pamela R. Pretorius*[1], *Stephanie L. Lerach*[1], *Jamie L. Lohr*[1,2], *Betsy Hirsch*[3], *Lisa A. Schimmenti*[1,2]. 1) Department of Pediatrics, University of Minnesota, Minneapolis, MN; 2) Lillehei Heart Institute, University of Minnesota, Minneapolis, MN; 3) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.
Congenital heart malformations are among the most common birth defects. A newborn was diagnosed with a Type A interrupted aortic arch and ventricular septal defect (VSD). This translational project was undertaken to identify the functional significance of a de novo *PTPN12* partial deletion (exons 3 through 18) that was identified in this patient. *PTPN12*, a known downstream target of the Ras pathway, has been associated with endothelial cell adhesion and migration. Zebrafish were used to model the pathogenicity of *PTPN12* loss and its effects on cardiovascular development. Gene knockdown in zebrafish was performed with an antisense oligonucleotide (Morpholino) to establish phenotypes associated with the loss of *ptpn12*. Observable effects of knockdown in zebrafish were analyzed using brightfield microscopy at 2 and 4 days post fertilization (dpf). Dose dependent phenotypes include dorsalization, pericardial edema, and hypopigmentation. These phenotypes are consistent with published data demonstrating expression in the head, branchial arches, and tail. Viable morphants were grouped into three classes based on the severity of these phenotypes. The development and structure of the great vessels and surrounding vasculature were evaluated using transgenic zebrafish strains and microangiography with a FITC conjugated dextran. Following fluorescent microscopy at 2 and 4 dpf, morphants demonstrated abnormal branchial arch and tail vasculature patterns as well as reduced blood flow. Tail vasculature patterns were further assessed, focusing on the dorsal longitudinal anasomatic vessel (DLAV), dorsal aorta (DA), and intersegmental vessels. Given the co-occurrence of the partial deletion of *PTPN12*, Type A interrupted aortic arch and VSD in the patient, and vascular phenotypic consequences of *ptpn12* knockdown in zebrafish, it is likely that *PTPN12* has a significant role in cardiovascular development and vessel formation. This represents a proposed novel function for this gene.

139. Proliferation dynamics of cardiomyocytes during atrial morphogenesis. ***Matthew Foglia***, *Ken Poss.* Department of Cell Biology, Duke University, Durham, NC.
During cardiac morphogenesis, the embryonic heart changes in structure from a continuous linear tube to a multi-chambered, valved pump composed of one atrium and one ventricle. Embryonic cardiomyocytes whose progeny will contribute to an atrium or the ventricle are specified early in development. How these cell populations change over time to produce chambers of distinct size and function is not well understood. In zebrafish, the ventricular wall thickens during juvenile growth, the result of dynamic proliferative events by a small number of dominant cardiomyocytes. By contrast, we have observed that the atrial wall remains a monolayer of cardiomyocytes throughout life. To define the patterns of cardiomyocyte division during atrial morphogenesis, we have traced the clonal progeny of embryonic atrial cardiomyocytes from embryogenesis to maturity using multicolor genetic labeling. Our experiments indicate that patterns of atrial cardiomyocyte division differ markedly from those of ventricular cardiomyocytes, explaining at least in part the different chamber morphologies. These experiments stand to shed light on a poorly understood aspect of chamber morphogenesis and provide a foundation for interpreting molecular mechanisms of heart development.

140. The bHLH transcription factor Twist1a functions to limit cardiomyocyte production. ***Kristina M. Garske***, *Yocheved L. Schindler*, *Deborah L. Yelon.* Division of Biological Sciences, University of California, San Diego, La Jolla, CA.
The production of the correct number of cardiomyocytes is crucial for the proper formation of the heart, as too few or too many cells can lead to a dysfunctional organ. Elucidating the network of transcription factors that dictate how many cardiomyocytes are produced is therefore a high priority for understanding the mechanisms underlying cardiac development and disease. Here, we show that the bHLH transcription factor gene *twist1a* limits cardiomyocyte production in the zebrafish embryo. Reducing the function of *twist1a* with anti-*twist1a* morpholinos (MOs) results in an increased number of cells expressing myocardial markers. This phenotype is a striking contrast to the severe reduction in cardiomyocyte production observed in mutant embryos lacking the bHLH transcription factor gene *hand2*. This opposition is reminiscent of the genetic antagonism observed between *Twist1* and *Hand2* in the mouse limb, leading us to consider whether genetic interaction between *twist1a* and *hand2* influences cardiomyocyte production. Interestingly, *twist1a* knockdown does not increase the production of differentiated cardiomyocytes in *hand2* null mutants, suggesting that Twist1a activity depends upon the presence of Hand2. To evaluate the cellular mechanism of Twist1a function, we investigated the origins of the excess cardiomyocytes found in *twist1a* morphants - do these cells result from increased specification or increased proliferation? EdU incorporation assays suggest that *twist1a* limits the proliferative capacity of cardiac progenitors, and *twist1a* morphants exhibit an expanded progenitor population, marked by *nkx2.5* and *hand2*. Together, our data indicate a previously unrecognized role for *twist1a* in inhibiting the production of cardiomyocytes from the cardiac progenitor pool and suggest that this function is dependent on, and potentially upstream of, *hand2* function.

141. Small Molecule Screening of Zebrafish Models of Disease and Development. ***Sarah Baxendale***, *Tanya T Whitfield, Vincent T Cunliffe, Steve A Renshaw, Freek van Eeden, Marysia Placzek.* Bateson Centre Screening Unit, Department of Biomedical Science, University of Sheffield, Sheffield, South Yorkshire, United Kingdom.
The size, transparency, fecundity, absorption characteristics and rapid development of the zebrafish embryo make this organism an attractive vertebrate model system with which to undertake drug screening. We have established a facility for medium-throughput screening using high content biological assays with the dual aim of identifying novel research tools and the establishment of systems to speed the identification of hits and generation of lead compounds for drug discovery. The Bateson Centre Screening Unit is able to provide a variety of whole-organism screening methods, including a range of different morphological, behavioural, enzymatic and fluorescent assay outputs, using a combination of automated and manual techniques. Research Groups within the Bateson Centre have established a number of disease models and assays that provide a basis for such high content molecular screens. To date, screens for ten different zebrafish assays have been completed, and verified hits have been obtained for each of these assays.

142. Swimming Pattern Alterations Induced by Environmental Estrogenic Compounds in Adult Zebrafish (Danio rerio). ***Basavaraj B Goundadkar****, Pancharatna A Katti.* Dept of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India.
Environmental estrogenic chemicals (EECs) are potential modulators of endocrine system causing alterations in the morphology, physiology, development, reproduction, homeostasis and behavior. The present study is an attempt to investigate the effects of EEC's on the swimming behavior of adult(body size: 2.5 ± 0.5 cm)zebrafish, Danio rerio. Adult zebrafish (n=20) were exposed to environmental relevant concentrations of environmental xenoestrogens (i) ethynilestradiol -EE2(5ng/L) (ii) diethylstilbestrol - DES (5ng/L) (iii) Atrazine - ATR (5mg/L) and (iv) bisphenol A - BPA (5mg/L) for two weeks. Corresponding controls were maintained. EECs were applied on alternate day after every water change. All experimental fish were fed on commercial pellets twice a day. Swimming parameters such as, (I) total time spent in swimming activity (ii)distance covered in vertical swimming (top-bottom direction) (iii) distance covered in horizontal direction (iv) latency to enter the top surface (v) erratic movements encountered and (vi) freezing bouts were analyzed by carefully reading the video clippings recorded at the end of the experiment, individually for each fish. Fish belonging to control group swam along the wall of the aquarium with a steady pace, frequent turns and stops, and occasionally short freezing episodes.Fish exposed to EE2 spent longer time at the bottom of the tank and did showed relatively less turns, stops and freezing episodes compared to controls.Fish exposed to DES, swam in a steady pace, in long sweeps of lower speed, and spent greater time in swimming at the surface water. Exposure to Atrazine and BPA caused faster swimming along with frequent turns and short freezing episodes. These results suggest that estrogenic compounds when present in surrounding water may alter swimming patterns/ behavior of fish fauna.

143. nlz1 is required for cilia formation in zebrafish embryogenesis. ***Sunit Dutta****, Shahila Sriskanda, Elangovan Boobalan, Ramakrishna Alur, Brian Brooks.* OGVFB, NEI/NIH, Bethesda, MD.
The formation of cilia is a fundamental developmental process affecting diverse functions such as intracellular signaling, mechanotransduction, tissue morphogenesis and body patterning. However, the mechanisms of ciliogensis during vertebrate development are not fully understood. In this report we describe a novel role of the Nlz1 protein in ciliogenesis. We demonstrate morpholino-mediated knockdown of nlz1 in zebrafish caused abnormal specification of the cells of Kupffer's vesicle (KV); a severe reduction of the number of cilia in KV, the pronephros, and the neural floorplate; as well as a spectrum of later phenotypes reminiscent of human ciliopathies. nlz1 is expressed in KV, in the nucleus of dividing cells and in the ciliary basal body of quiescent IMCD3 cells. In vitro and in vivo data indicate that Nlz1 acts downstream of the ciliary "master transcription factor", Foxj1 and, Nlz1, is upregulated by canonical Wnt signaling. Together, our data suggest a novel role of nlz1 in ciliogenesis and the morphogenesis of multiple tissues..

144. Zebrafish Pronephros Tubulogenesis and Maintenance of Epithelial Identity are Reliant on the Polarity Proteins Prkc iota and zeta. ***Gary F. Gerlach****, Rebecca A. Wingert.* Biological Sciences, The University of Notre Dame, South Bend, IN 46556, USA.
The zebrafish pronephros provides an excellent *in vivo* system to study the mechanisms of vertebrate nephrogenesis. When and how renal progenitors in the zebrafish embryo undergo tubulogenesis to form nephrons is poorly understood, but is known to involve a mesenchymal to epithelial transition (MET) and the acquisition of polarity. Here, we determined the precise timing of these events in pronephros tubulogenesis. As the ternary polarity complex is an essential regulator of epithelial cell polarity across tissues, we performed gene knockdown studies to assess the roles of the related factors atypical protein kinase C iota and zeta (*prkci, prkcz*). We found that *prkci* and *prkcz* serve partially redundant functions to establish pronephros tubule epithelium polarity, and that the loss of *prkci* or both *prkci/z* disrupted renal morphogenesis, the latter likely due to cardiac defects that prevent normal fluid flow to the kidney. Surprisingly, tubule cells in *prkci/z* morphants displayed ectopic expression of the transcription factor *pax2a* and several podocyte-associated genes, including *wt1a*, *wt1b*, and *podxl*, suggesting that *prkci/z* are necessary to maintain renal epithelial identity. Knockdown of genes essential for cardiac contractility and vascular flow to the kidney, such as *tnnt2a* and/or elimination of pronephros fluid flow through knockdown of the intraflagellar transport gene *ift88*, was not associated with ectopic pronephric gene expression. This suggests a unique role for *prkci/z* in tubule epithelial identity separate from the consequence of disruptions to renal fluid flow. Interestingly, knockdown of *pax2a*, but not *wt1a*, was sufficient to rescue ectopic tubule gene expression in *prkci/z* morphants. These data suggest a model in which the redundant activities of *prkci* and *prkcz* are essential to establish tubule epithelial polarity and also to maintain proper epithelial cell-type identity in the tubule by inhibiting *pax2a* expression. These studies provide a valuable foundation for further analysis of MET during nephrogenesis, and have implications for understanding the pathways that affect nephron epithelial cells during kidney disease and regeneration.

145. The Balbiani body proteome in zebrafish. ***A. Jamieson-Lucy****, M. C. Mullins.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
The Balbiani body is the first marker of polarity in vertebrate oocytes and presents a novel mechanism generating cell polarity. During early zebrafish oogenesis, the Balbiani body carries localized mRNAs and proteins from the nucleus to the vegetal cortex, polarizing the oocyte. However, little is known about the Balbiani body, and the mechanism behind Balbiani body-dependent oocyte polarity cannot be explained by functional analysis of its few known protein components. We isolated Balbiani bodies and performed mass spectrometry to define the Balbiani body proteome. Using this approach, we identified over 70 proteins within the isolated Balbiani body sample, including elements of the cytoskeleton and RNA binding proteins. Obtaining these targets is a prerequisite for understanding the action of the Balbiani body at a protein level. Furthermore, these results show that the Balbiani body is a complex structure that requires many proteins working in concert to initiate oocyte polarity.

146. Analysis of the Maternal- Effect Mutations that Affect Nuclear Dynamics at Fertilization. ***Ashley A Baldo****, Francisco Pelegri.* Endocrinology and Reproductive Physiology, University of Wisconsin-Madison, Madison, WI.
Homozygosity for maternal-effect mutations results in phenotypically normal mothers that exhibit a mutant phenotype in their offspring despite sperm-derived DNA. This is because early developmental processes rely on genes active during oogenesis, which deposit products

in the oocyte. Many of these mutations have been found in zebrafish such as *janus, nebel, ichabod,* etc. Two, *futile cycle* (*fue*) and *motley* (*mot*), affect pronuclear dynamics at fertilization.
fue embryos do not undergo pronuclear congression, but undergo cell division. Past work identified the gene required for pronuclear congression as *lymphoid restricted membrane protein* (*lrmp*), also called *jaw1*. Lrmp protein and *lrmp* mRNA localize to the spindle in cell cycle-dependent patterns that suggest coordination of translation and protein transport to the nuclear envelope. To learn how this protein is translationally regulated in early embryos, we have designed antibodies against various regions of Lrmp to monitor specific regions of the protein as it is translated during the cell cycle. Also, we are initiating screens for Lrmp protein interactors using a yeast two-hybrid system. Lastly, Lrmp homologues are found in all vertebrates except for rodents and we are exploring the expression of this protein in other vertebrate species.
mot embryos cannot undergo cell division. This prevents the extrusion of the polar body during meiosis II, which occurs in zebrafish post-fertilization. Past work identified the mutated gene as *birc5b*, a homolog of mammalian Birc5b/Survivin. In wild-type, the polar body DNA condenses, but the oocyte DNA decondenses in anticipation of pronuclear fusion. However, in *mot* mutants, both polar body and oocyte DNA undergo condensation; suggesting that DNA condensation signals exist and are improperly segregated in the absence of polar body extrusion. Using immunofluorescence and electron microscopy we are analyzing polar body and female pronuclei condensation in wild-type and mutant embryos. Also, markers for the midbody complex are being used to study the formation and the segregation of this structure during meiosis.

147. The role of Cdx transcription factors in spinal cord formation. ***Alana Beadell***[1], *Jessie Chang*[2], *Adam Kuuspalu*[1], *Robert Ho*[1,2]. 1) Department of Organismal Biology and Anatomy, University of Chicago; 2) Committee on Development, Regeneration and Stem Cell Biology, University of Chicago.
The spinal cord is a unique vertebrate feature originating from the embryonic neural plate as the most posterior aspect of the nervous system. In zebrafish, and perhaps all chordates, Cdx transcription factors are key determinants of the spinal cord region. Cdx/Caudal homologs are expressed in the posterior tissues of every major group of bilaterians and control the sequential addition and identity of body segments in the developing trunk and tail. Additionally in vertebrates, cdx genes are among the earliest expressed in the presumptive spinal cord territory. Loss of Cdx function in zebrafish causes a posterior expansion of the hindbrain and a concomitant elimination of the spinal cord territory, while overexpression of Cdx causes an anterior expansion of the spinal cord at the expense of hindbrain. It is not yet known how Cdx acts to specify spinal cord fate, or whether its role in axial elongation is causally connected to or independent of its nervous system function. The posterior Hox genes are known Cdx targets and are key effectors of Cdx action in the presumptive spinal cord, but there may be Hox-independent Cdx-mediated mechanisms operating as well. Previously, a wild-type versus Cdx loss-of-function microarray experiment was performed in our laboratory to identify gene products down-regulated in whole embryos in the partial absence of Cdx. We have used this data to identify genes that may mediate Cdx action in the presumptive spinal cord by in situ hybridization. Some candidates are specifically expressed in spinal cord proneural cells in wild type embryos and thus likely act downstream of spinal cord specification. Others exhibit expression within the posterior neural plate and/or tailbud at late gastrulation/early somite stages in wild type embryos, but not in Cdx morphants, and thus may more directly control spinal cord specification. Follow-up experiments will include the phenotypic characterization of embryos in which gene products regulated by Cdx are knocked-down and/or over-expressed to ascertain their consequences for spinal cord development and axial elongation.

148. moved to presentation #20 *cdc25a* times the cell cycle to facilitate mesodermal cell differentiation during posterior body formation in zebrafish. ***Cortney Bouldin***[1], *Corey Snelson*[1], *Gist Hank Farr*[1,2], *David Kimelman*[1]. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA.
During the early stages of vertebrate body formation, an embryo grows from the head to the tail to generate the anterior-posterior (AP) axis. Throughout embryo elongation, the hind end of the embryo maintains a population of undifferentiated cells that provides the raw cellular material for posterior body formation and a population of differentiating cells that will contribute directly to the growing AP axis. As elongation is completed, the numbers of undifferentiated cells are winnowed to depletion. To prevent premature depletion of the undifferentiated cells, both the numbers and the rate of release must be kept in balance until the AP axis is finished. At the onset of this study, we hypothesized that careful control of proliferation in the undifferentiated cells is critical for the completion of the vertebrate body. Through single cell fate-mapping experiments, we have found that proliferation in the undifferentiated cells can be divided into an early-rapid phase followed by a late-quiescent phase. The two phases of proliferation are matched by expression of the mitotic phosphatase, *cdc25a*. In undifferentiated cells, *cdc25a* is expressed during the early-rapid phase followed by depletion of *cdc25a* during the late-quiescent phase. Further by misexpressing *cdc25a* during the late-quiescent phase, we have found that if expression of *cdc25a* and proliferation are not restricted the ability of undifferentiated cells to proceed through mesodermal differentiation and contribute to the AP axis is blocked.

149. Roles of intracellular calcium mobilization in intercellular signaling, cell-type specification, and tissue patterning. ***Alexis Chagovetz***, *Derrick Gunther, Mick Jurynec, Dana Klatt, David Grunwald.* Dept of Human Genetics, University of Utah, Salt Lake City, UT.
The Ryanodine Receptor (RyR) intracellular calcium release channels (CRC) regulate release of calcium from intracellular stores in the ER/SR. Though expressed in many cell types, they are best known for their roles in muscle contraction; indeed loss-of-function mutations are associated with myopathy in humans. We propose this narrow view of the RyR function fails to account for the full range of phenotypes observed in humans carrying RYR1 mutations, which include slow muscle cell defects. Here we elucidate unexpected functions of RyR in the zebrafish embryo. We demonstrate that RyR function is needed for asymmetric expression of southpaw and subsequent left/right patterning. Further we show multiple Hedgehog-dependent cell specification events, including generation of somite muscle cells and dorsal

root ganglia, require RyR-mediated calcium mobilization. With blocked RyR function, adaxial precursor cells develop into fast muscle cells, as they would in the absence of Hh signaling. We present these findings and report the results of our efforts to identify the specific combinations of ryr genes that support these developmental functions. We have i) reinvestigated the expression of ryr genes, ii) generated null ryr alleles, and begun to analyze mutant embryos carrying combinations of the mutations.

150. Characterization of syndecan-3 function during the early developmental embryonic stage from zebrafish. *Chih-Ming Chou*[1], *Gen-Der Chen*[3], *Yi-Chung Chen*[4], *Cheng-Jen Huang*[3], ***Chih-Ming Chou***[1,2]. 1) Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan; 2) Department of Biochemistry, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan; 3) Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; 4) Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan.
The syndecan proteoglycans are a class of receptor, bearing heparan sulfate chains that interact with numerous ligands including growth factors, morphogens, and extracellular matrix molecules. Syndecans have been suggested to function as co-receptors with other signaling receptors, such as FGF receptors and integrins. In invertebrates, syndecans are mainly associated with growth factor interactions rather than cell adhesion. Syndecan-3 (N-syndecan) is mainly expressed in the nervous system. It has been suggested to function in cell adhesion, neurite guidance, and cell migration during development of the nervous system. Only three syndecan homologues are present in the zebrafish genome, these are syndecan-2, -3, and -4. However, little is understood in terms of its role in development. In this study we investigated the expression of syndecan-3 (zSyn3) and its role on zebrafish morphogenesis at early developmental stage. By using RT-PCR and whole-mount in situ hybridization analysis, the data shown that the zebrafish zSyn3 mRNA was expressed ubiquitously and abundantly at 36 hpf during the developmental stages and mainly expressed at adult brain, eye and intestine tissues. To explore the in vivo function of zSyn3 during zebrafish developmental stage, we used morpholino knockdown approach and found that zSyn3-MO knockdown morphants displayed mild posterior mesodermal defect and severe posterior mesodermal defect with eye and brain mutant. Expression analysis of the vega1 (vox), chordin (chd), and no tail (ntl) by whole mount in situ hybridization in zSyn3-MO knockdown morphants the data shown these malformations were caused by altered chd and ntl expression but not vox during early developmental stage. We suggest the zSyn3 was coordinated Wnt and Fgf signaling for formation of the posterior body and skeletal muscle development in zebrafish.

151. Analysis of zebrafish *eftud2* function in ocular and craniofacial development. ***Brett Deml***[1,2], *Elena Semina*[1,2]. 1) Department of Pediatrics, MCW, Milwaukee, WI; 2) Department of Cell Biology, Neurobiology and Anatomy, MCW, Milwaukee, WI.
Anophthalmia and microphthalmia (A/M) are congenital disorders defined as the absence or reduction in size of the eye with one-third of cases being syndromic. Approximately 60% of patients with A/M lack a molecular diagnosis. Using whole exome sequencing we identified a mutation in *EFTUD2* in a patient with syndromic microphthalmia. Mutations in *EFTUD2*, a U5 small nuclear ribonucleoprotein, have previously been associated with mandibulofacial dysostosis and microcephaly; microphthalmia has not been reported. The purpose of this study was to investigate the role of zebrafish *eftud2* in eye development through analysis of its expression and the development of an *eftud2* knockout zebrafish line. In situ hybridization using an anti-sense RNA probe was performed to examine the expression pattern of *eftud2*. TALEN pairs were constructed following the Sanjana et al. 2012 protocol. TALENs targeted exon 5 of *eftud2* and are predicted to abolish a BclI restriction site. Zebrafish embryos were injected with TALEN RNA at the 1-4 cell stage and sacrificed at 24-hpf to determine cutting efficiency. *eftud2* in situ shows broad expression throughout the embryo; however, higher level of expression can be observed in the developing head and eye. At 18- and 24-hpf stronger expression can be seen throughout the brain and retina. By 48-72 hpf, increased level of *eftud2* transcript can be detected in the ciliary marginal zone (CMZ) of the retina. BclI digestion analysis of 16 *eftud2* TALEN-injected larvae demonstrated interruption of the restriction site in all embryos. The TALEN-injected embryos were raised to adulthood and crossed; ~15% of the produced embryos carried genomic mutations, all of which were truncation alleles. These F1 fish were raised to adulthood and genotyped; generation and analysis of the embryos produced by *eftud2* heterozygous crosses are underway. The observed pattern of zebrafish *eftud2* expression suggests a possible role in eye development, with expression in the CMZ possibly indicating a role for *eftud2* in eye size determination. Efficient TALEN pairs for disruption of zebrafish *eftud2* were generated with phenotypic analysis still ongoing.

152. *Nr2f1a* Act Downstream of RA Signaling to Promote Pharyngeal Muscle at the Expense of Ventricular Cardiomyocytes in Zebrafish. ***Tracy Dohn***[1,2], *Joshua Waxman*[2]. 1) Molecular and Developmental Biology, College of Medicine, University of Cincinnati; 2) Cincinnati Children's Hospital, Cincinnati, OH.
Many developmental syndromes have both cardiac and pharyngeal defects suggesting similar molecular mechanisms may control the development of these progenitor populations. Furthermore, recent studies have indicated that cardiac and pharyngeal muscle (PM) share common progenitors. However, upstream pathways that control the cardiac and PM progenitor fate decisions are not understood. Retinoic acid (RA) signaling is required for proper cardiac and PM development indicating it is a candidate to direct fate decisions between these progenitors. We identified *nr2f1a* as a target of RA signaling in the anterior lateral plate mesoderm (ALPM). Interestingly, *nr2f1a* depletion leads to increased ventricular cardiomyocyte (VC) number, while RA signaling depletion leads to an increase in both atrial and VC number. To determine the origin of the excess VCs in *nr2f1a* deficient embryos, we performed *in situ* hybridization and fate-mapping experiments. We found a posterior expansion of VC progenitors (VPs) within the ALPM suggesting a possible fate transformation with adjacent mesodermal cell types. To determine if other ALPM populations were affected in *nr2f1a* deficient embryos, we examined other mesodermal outcomes in our fate maps and with immunohistochemistry. We found a loss of PMs comparable to that found in RA signaling deficient embryos, while pharyngeal endothelial cells were not affected. Blastula transplantation analysis indicates *nr2f1a* acts cell autonomously to direct VP and PM specification, supporting that it regulates a fate decision between VC and PM progenitors (PMPs). Interestingly, when we examined the PMP markers *tbx1*, *tcf21*, and *six1b*, they were only slightly decreased, while *Tg(tcf21:GFP)* zebrafish still show *tcf21+* cells contributing to the remaining PMs in *nr2f1a* deficient embryos. Together, our data suggest that in the

ALPM, *nr2f1a* acts downstream of RA signaling to promote PMP specification at the expense of VPs through a novel *tbx1/tcf21*-independent pathway.

153. The conserved transcription factor grainyhead-like 3 regulates multiple aspects of embryonic development. ***Sebastian Dworkin***[1], *Charbel Darido*[1], *Darren Partridge*[1], *Smitha Georgy*[1], *Tomasz Wilanowski*[5], *Graham Lieschke*[3], *Joan Heath*[2], *Stephen Jane*[1,4]. 1) Department of Medicine, Monash University, Melbourne, Victoria, Australia; 2) Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; 3) Australian Regenerative Medicine Institute, Monash University, Melbourne, Victoria, Australia; 4) Alfred Hospital, Melbourne, Victoria, Australia; 5) Laboratory of Signal Transduction, Nencki Institute of Experimental Biology, Warsaw, Poland.
Each year, ~4% of children are born with some form of birth defect, primarily caused by aberrant gene expression during embryogenesis. Transcription factors coordinate developmental gene expression and generally fall into two classes - those which specify patterning down a particular tissue lineage and those which regulate basic cellular processes, such as cell growth, cell survival, proliferation or apoptosis, across multiple lineages. Far rarer, however, are those transcription factors which bridge this divide, by not only influencing the development of a number of different tissues, but do so through the independent regulation of multiple cellular processes. One such family of transcription factors are the Grainyhead-like (Grhl) genes, vertebrate orthologues of the fruit-fly (Drosophila) gene “grainyhead” (grh). Using Drosophila and mouse models, we and others have shown that grh/Grhl-genes (Grhl1-3) are responsible for craniofacial development, gastrulation, skin-barrier development, dorsal hole/neural tube closure, wing/cochlear hair orientation, cell migration, survival and cytoskeletal polarity. Using the zebrafish, we have further shown that one of these orthologues, grhl3, regulates convergence-extension movements during gastrulation, growth and development of the craniofacial skeleton, and shaping of the midbrain-hindbrain boundary (MHB). We have shown that precise temporal and spatial regulation of grhl3 is essential for correct function - even slight perturbations in expression have drastic developmental consequences. Our over-arching aim is therefore to identify which grhl3 target genes are regulated in these contexts and to determine whether a “core” set of targets underpins each defect.

154. Gradual recruitment and selective clearing generate germ plasm aggregates in the zebrafish embryo. ***Celeste C. Eno***, *Francisco J. Pelegri*. Genetics, University of Wisconsin, Madison, WI.
Determination of primordial germ cells (PGCs) is one of the earliest decisions in animal embryogenesis. In many species (including zebrafish), PGCs are determined through maternally-inherited germ plasm ribonucleoparticles (RNPs). RNPs are transmitted as dispersed molecules during oogenesis, which after fertilization multimerize and become recruited as large aggregates at furrows for the first and second cell cycles. We also show that germ plasm RNPs are recruited during the third cell cycle, but only transiently. Our imaging and analysis data support a mechanism in which systematic local gathering of germ plasm RNPs during cytokinesis and threshold-dependent clearing contribute to forming germ plasm aggregates with the highest RNP number and germ cell-inducing potential. Multiple maternal-effect mutants have irregular germ plasm aggregation, allowing for the study of the mechanisms by which aggregation, recruitment and compaction occur. Germ plasm does not aggregate or recruit to the furrow in *aura*/Mid1ip1L mutant embryos, and in *nebel*/Aspdh mutant embryos, RNPs do not distally compact. *aura* embryos do not have the characteristic long f-actin bundles, which we hypothesize are necessary for aggregation. Whereas, *nebel* mutant embryos do not display the characteristic slow calcium waves, which are present in the first two cleavage furrows, and we hypothesize the slow calcium waves directs distal compaction of RNPs. Maternal-effect mutants and drug inhibitors allow for the effective study of proper germ plasm RNP aggregation, compaction and eventual PGC formation.

155. Dissecting the *cis*-Regulatory Logic of the Periderm Gene Regulatory Network. *Byron Williams*[1], *Kaylia Duncan*[2], *Danielle Beekman*[1], *Gregory Bonde*[2], ***Albert Erives***[1], *Robert Cornell*[2]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA.
When it comes to developmental genetic disorders, it is more technically correct, strategically practical, and clinically efficacious to focus on transcriptional enhancers rather than gene loci as the causative heritable agents of disease for three reasons. First, transcriptional enhancers determine a gene’s myriad specificities for cell-type, tissue, and physiological response. Second, post-genomic studies have shown that enhancers are diffusely distributed around the transcript-based definition of a gene locus with several other genes interposed between them and their target genes. Third, sublethal mutations at enhancers are more heritable and numerous than lethal protein-coding mutations. We are applying these post-genomic perspectives to human developmental genetic disorders of the epidermis. Epithelial cells of the epidermis, including the oral epithelium, form the primary barrier between the multicellular body and the environment. Accordingly, mutations in genes underlying epidermal differentiation lead to several human birth defects that range from aesthetic disfigurement to life-impacting morbidity. To accomplish this goal, we are dissecting the homologous Gene Regulatory Networks (GRNs) in teleost fish by identifying all relevant transcriptional enhancers, their internal structure (binding site composition and *cis*-regulatory logic), their target genes, and their target genes’ role in the GRN. Using the periderm transcriptome, ChIP-seq data sets for relevant transcription factors (TFs) and chromatin marks, comparative enhancer genomics, and *in vivo* transgenic analyses of predicted enhancers, we are reconstructing the architecture and connectivity of the periderm GRN. We started by identifying enhancers of *interferon regulatory factor* 6 (*irf6*). Human *IRF6* is among the genes implicated in human genetic disorders causing cleft lip and cleft palate, as it is responsible for Van der Woude syndrome. We summarize our computational approach, and report on our initial Periderm Enhancers and how they advance our understanding of this important GRN.

156. microtubule-actin crosslinking factor (Macf1), A Maternal-Effect Gene That Functions In Animal-Vegetal Polarity Establishment In Zebrafish Oocytes. ***Matias Escobar-Aguirre***, *Ricardo Fuentes, Mary Mullins.* University of Pennsylvania , Philadelphia, PA.
In zebrafish oocytes, animal-vegetal polarity (A-V) is established through formation and translocation of the Balbiani Body (Bb) to the prospective vegetal pole of the oocyte. The Bb is a highly conserved structure present from insects to humans that contains mitochondria,

ER, RNAs and proteins. Importantly, animal-vegetal oocyte polarity also determines the embryonic anterior-posterior axis in zebrafish, Our lab has demonstrated that the establishment of oocyte animal-vegetal polarity depends on the function of the bucky ball and macf1 genes, which are required for Bb formation and disassembly, respectively. In the macf1 mutant, as in normal oocytes, RNAs of different germ cell determinants localize to the Bb. However, as stage I of oogenesis progresses in macf1 mutants, the Bb enlarges and never reaches the vegetal cortex. At this stage, RNAs that normally localize to the Bb can be seen disorganized and occupy a broad area in the oocyte cytoplasm. Additionally, macf1 mutants display an asymmetrically-positioned nucleus. Macf1 is a spectraplakin that connects cytoskeleton components to functionally integrate them. We are elucidating the molecular mechanism by which Macf1 interacts with the cytoskeleton to maintain Bb structure and translocate it to the vegetal pole. By performing in situ hybridizations of germ cell determinants localized to the animal-vegetal pole and immunostaining to visualize cytoskeletal components, along with functional experiments to assess the effect of disrupting the cytoskeleton on Bb structure and nuclear positioning, I am investigating the Macf1-dependent mechanism that integrates cytoskeletal dynamics to function in maintaining Bb structure, movement and in positioning the nucleus in oocytes. These studies are expected to elucidate how Macf1-mediated cytoskeleton interactions lead to A-V polarity establishment during early zebrafish oogenesis.

157. Biological effect of Lysophosphatidic Acid Signaling in the Embryonic Development of the Zebrafish. ***Frisca Frisca***[1], *Alice Pébay*[1], *Yona Goldshmit*[1,2], *Celia Vandestadt*[2], *Jan Kaslin*[2]. 1) Department of Opthalmology,Centre for Eye Research Australia, University of Melbourne, East Melbourne, Victoria, Australia; 2) Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria, Australia.
In vertebrates, the axis mesoderm, which constitutes midline structure of the body axis, is one of the crucial body parts during development. It has various functions including to provide structural support and as a barrier for the establishment of Left-Right (L-R) asymmetry of the internal organ in the zebrafish embryo. Lysophosphatidic acid (LPA) is a signaling phospholipid that induces pleiotropic effects in many cell types, mainly through binding its specific G-protein coupled receptors. While major players of embryonic development processes have been associated to diverse signaling pathways, the role of phospholipid signaling in this context remains understudied. Here, we assessed the role of LPA in the embryonic development of the zebrafish. We observed embryonic axis mesoderm defects in a dose-dependent manner following the gain of function of LPA signaling which is suggested due to an impaired cells migration during gastrulation. This was accompanied by the mis-expression of asymmetric nodal-related genes in the lateral plate mesoderm during mid somitogenesis, indicating the perturbed L-R patterning of the internal organ of the embryo. Blocking lpa_{1-3} receptors using the antagonist Ki16425 rescued this phenotype, demonstrating the involvement of LPA receptor in this biological effect. This was further supported by the expression profiles of lpa_{1-3} during the early axis mesoderm formation. Taken together, our data shows for the first time the unique role of LPA signaling, through lpa_{1-3}, in regulating axis mesoderm formation as well as the establishment of the L-R asymmetry in the early embryo. This study thus highlights the unique role of phospholipid signaling during embryogenesis.

158. The role of Gas6 and Axl in hindbrain segmentation and neuronal patterning in zebrafish. ***Priyanjali Ghosh***, *Charles Sagerstrom.* UMASS Medical School, Worcester, MA.
In zebrafish, hoxb1b controls rhombomere (r) formation and it upregulates downstream targets like Eph receptors and their ephrin ligands, growth arrest specific gene 6 (Gas6) and the receptor tyrosine kinase Axl. Eph-ephrins play a key role role in hindbrain boundary formation. Infact, embryos injected with EphA4a (r3/5) and ephrinb2a (r4) antisense mopholinos show a disrupted r4 boundary (Moens, 2005). Little is known about Gas6 and Axl in the zebrafish hindbrain; however, they share similarities with the Eph-ephrins. The X-ray crystal structure of the receptor ligand complex of Eph-ephrins and Gas6-Axls are highly comparable (Himanen, 2001; Sasaki, 2006). We have found that like the Eph-ephrins, Gas6 is expressed downstream of the transcription factor Valentino. Using RNA In situ-hybridization, we also show a rhombomere restricted expression pattern of Gas6 (r5/6) and Axl (r1/2 to r4), showing their presence at the r4/5 boundary. These similarities are suggestive of Gas6-Axl having a putative role in boundary formation. Hindbrain segmentation sets stage for the formation of the neuronal circuit. Transcription factors and environmental signals acting as chemoattractants regulate the proper development and migration of neurons. Along with boundary formation, Eph-ephrins also mediate axonal pathfinding. Misregulation of EphA4a-ephrinb2a expression causes defective axonal routing in the zebrafish forebrain (Bonkowsky, 2012). Research in human and rat shows Gas6 as a novel chemoattractant that induces cytoskeletal reorganization via the phosphorylation of Axl leading to chemotaxis of vascular smooth muscle cells and Gonadotropin releasing hormones neurons (Liu, 1998; Nielsen-Preiss,2007). These finding lead us to hypothesize that Gas6-Axl play a role in neuronal patterning. Using anti-sense morpholinos against Gas6 and Axl we plan to look for potential defects in hindbrain segmentation and neuronal patterning. We are also using the CRISPR/cas9 system to create EphA4a, ephrinb2a, Gas6 and Axl knockout lines. Insofar, we have successfully created a mutation in the EphA4a gene and we are raising fish to screen for founders. These studies will elucidate the functions of Gas6 and Axl in the zebrafish hindbrain.

159. The V-ATPase accessory protein Atp6ap1b mediates dorsal forerunner cell proliferation and left-right asymmetry in the zebrafish embryo. ***J. J. Gokey***, *A. Dasgupta, J. D. Amack.* Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY.
Left-right (LR) patterning of the early embryo is critical for subsequent development of asymmetric organs such as the heart. Small molecule screens have identified a role for the vacuolar-type H+-ATPase (V-ATPase) in establishing LR asymmetry, but exactly how V-ATPase activity impacts LR patterning remains unclear. The V-ATPase is a multi-subunit proton pump in membranes that maintain organelle and cytosolic pH by pumping protons into organelles and out of the cytoplasm. V-ATPase accessory proteins are thought to modulate V-ATPase activity, but little is known about these proteins during development. In zebrafish, the V-ATPase accessory protein Atp6ap1b is maternally supplied and prominently expressed in dorsal forerunner cells (DFCs) that give rise to the transient organ Kupffer's vesicle (KV). Motile cilia that project into the KV lumen generate an asymmetric fluid flow that is required for establishing LR asymmetry. Depletion of Atp6ap1b using morpholinos disrupted the formation of KV and altered LR asymmetry. Atp6ap1b knockdown significantly decreased the length and number of KV cilia and reduced KV organ size. These KV defects were rescued by ectopic Atp6ap1b expression.

Interfering with V-ATPase function resulted in similar KV and LR defects. To determine the cause of KV size and cilia defects, we analyzed behaviors of the precursor DFCs during gastrulation. Live imaging showed that while the movements and cohesion of DFCs was unaffected in Atp6ap1b depleted embryos, the number of DFCs was reduced. Molecular markers of cell division or apoptosis indicated Atp6ap1b mediates proliferation of DFCs during gastrulation. Interestingly, fluorescent pH indicators revealed a decrease in cytoplasmic pH—but not lysosomal pH—in DFCs relative to neighboring cells in live Atp6ap1b depleted embryos. These results uncover a role for Atp6ap1b in regulating the pH of DFCs and maintaining the proliferation rate of these precursors that is necessary to build a KV of the proper size with sufficient cilia to generate LR asymmetry in the embryo.

160. Heat shock sensitivity immediately after pronuclear fusion inhibits the second cell division and results in whole genome duplication. ***Jonathon A. Heier****, Kendra Takle, Francisco Pelegri.* University of Wisconsin-Madison, Madison, WI.
Ploidy manipulation is a useful genetic tool in many animal model systems, including the zebrafish. The heat shock method (heat shock at times 13-15 minutes post-fertilization) to inhibit cytokinesis of the first mitotic cycle, originally applied to zebrafish by Streisinger and colleagues, is particularly attractive because it results in whole genome duplication and allows for immediate and complete homozygosity. However, the yield of gynogenotes produced by this and other ploidy manipulation techniques remains relatively low, preventing their widespread use. Our previous studies on parental effects caused by mutations in the centrosomal component Sas-6 showed that aberrant centrosomal duplication results in a cell division stall of exactly one cell cycle without interfering with DNA replication, thus promoting whole genome duplication. We scanned the first hour after fertilization for a time period which, when used to apply a heat shock, would induce an exact one cell cycle division stall. Remarkably, a 2-minute heat pulse initiated at 22 mpf, corresponding to the time immediately following pronuclear fusion, results in a high (>80%) frequency of embryos exhibiting a precise one cell cycle stall. These embryos undergo normal cytokinesis corresponding to the first cell cycle (35 mpf), exhibit a cell division stall at the time corresponding to the second cell cycle (50 mpf) and continue to divide one cell cycle later to generate normally patterned embryos. Such embryos exhibit a duplicated set of chromosomes and are inviable, consistent with having tetraploid genetic content. Heat shock at later time points results in a variety of cell division defects and low viability, suggesting that the time period prior to the initiation of cell division is particularly favorable for ploidy manipulation. Coupled to haploid production through the use of UV-treated sperm, the skipping of the second cell division induced by heat shock at 22-24 mpf promotes homozygosity and gynogenetic development at improved rates compared to the standard (13-15 mpf) heat shock treatment. Gynogenetically derived embryos can become viable, fertile adults and we are currently in the process of developing lines using this method.

161. Transcriptional control of the lateral mesoderm patterning. ***C. Hess****, I. Meyer, C. Mosimann.* Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
The development of the heart, blood vessel, and blood are tightly synchronized in the forming embryo. Cardiovascular and blood cell lineages arise together in a common embryonic structure, the lateral mesoderm (LM). How the LM distinguishes itself from the remaining mesoderm and patterns into such diverse cell fates and remains poorly understood. Gene-regulatory sequences read out lineage-specific signaling and transcription factor codes and provide the key to understanding the combinations of molecular mechanisms that drive cell fates. Towards molecularly defining the specification of LM from general mesendoderm, we set out to isolate regulatory elements of genes specifically expressed in the early LM. We performed FACS-based isolation of LM cells from bud-stage zebrafish embryos transgenic for LM-marking GFP reporters and performed Affymetrix microarray analysis. This provided a unique gene list of approximately 250 genes up-regulated more than 3-fold in the LM that serves as a basis to uncover their LM-specific gene regulation. We are now systematically screening through candidate genes for their LM-specific enhancer elements, including hand2, tmem88a, pcdh12, and bmp6. We have isolated new candidate enhancers that drive transgenic reporter expression in subdomains of the LM or LM-derived organs. To elucidate the marked LM lineages, we compare the expression pattern of candidate enhancer transgenics with established transgenic reporters for the hemangioblast markers scl and lmo2 using SPIM, and are perturbing the developing LM using chemical modulators of key signaling pathways. Our preliminary data revealed that FGF signaling plays a temporally tightly regulated role in control of several early LM elements.

162. Efficient and simple method for targeted mutagenesis in medaka using TALENs and CRISPR/Cas system. ***Satoshi Ansai****, Masato Kinoshita.* Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.
Nuclease-mediated genome editing is rapidly becoming an essential technology for reverse genetics in a wide range of organisms. Here, we report efficient and simple methods for targeted mutagenesis in medaka using custom-designed transcription-activator like (TAL) effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system-based RNA-guided nucelases (RGENs). Firstly, we designed both TALENs and single guide RNAs (sgRNAs) for the medaka *DJ-1/park7* locus and injected into fertilized eggs as in vitro transcribed RNAs. All 12 screened RNA-injected fish transmitted mutations to progeny with high efficiency (44-100% in TALENs and 43-100% in RGENs), and then fish harboring a TALEN-induced frameshift mutation in both alleles lost DJ-1 protein. We have already examined that our designed TALENs and sgRNAs were capable of inducing mutations at other genomic loci with high success rate (38/43 in TALENs and 9/12 in sgRNAs). These result indicate that TALENs and RGENs work as efficient tools for targeted mutagenesis in medaka, in particular, RGENs will be widely used because of their easier customizing process than TALENs. However, we found that off-target alterations at two genomic loci were induced in the RGEN-injected embryos, suggesting that off-target effects should be considered in the analysis of genome-edited fish by RGENs. Additionally, we had developed a heteroduplex mobility assay (HMA) combined with an automated microchip electrophoresis system (HMA-MultiNA), which is a simple and high-throughput method for evaluation of in vivo activity of the nucleases and for genotyping mutant fish of F1 or later generations. We also found that a specific pattern of mutations is dominant for the nucleases harboring several base pairs of homologous sequences in their target sequence. These findings will provide helpful information for efficient and rapid genome editing using the nucleases.

163. SNPfisher: A Tackle Box of Tools for Catching Useful Genetic Variants in Laboratory Zebrafish. ***Matthew G. Butler***[1], *James R. Iben*[2], *Kurt C. Masden*[3], *Jonathan A. Epstein*[2], *Michael Granato*[3], *Brant M. Weinstein*[1]. 1) Program in Genomics of Differentiation, NICHD-NIH, Bethesda, MD; 2) Program in Developmental Endocrinology and Genetics, NICHD-NIH, Bethesda, MD; 3) Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.
Although a variety of different laboratory strains are commonly used in zebrafish research, the vast majority of the current zebrafish genome assembly (Zv9) was generated using sequence from one strain, Tübingen (Tü). The genetic variation between this reference sequence and other strains remains obscure and is not easily queried. Furthermore, despite reports of high estimates of intra- and interstrain variation in zebrafish, almost all sequence-based molecular biology reagents such as morpholinos, TALENs, or CRISPRs, are routinely designed solely from the available Tü sequence. We have used whole-genome sequencing to survey genetic variation in the tüpfel longfin (TL), WIK, and Ekkwill (EK) strains. Each population sample was sequenced to an average of 16X coverage. Importantly, we clustered our analysis by requiring a minimum read depth from each strain for a position to be included in our dataset. We organized our variation data into a user-friendly online database termed SNPfisher (snpfisher.nichd.nih.gov). The SNPfisher tool allows researchers to filter genetic variants in any region of interest by frequency, read depth, and/or strain. This tool also reports restriction enzyme changes (if any) caused by an observed variant as well as designs primers to amplify that variant. We also provide links to display our variation data in the UCSC genome browser. The SNPfisher site also contains a blat engine to quickly interrogate oligos for polymorphisms. To facilitate cloning by whole genome or exome sequencing, we also provide our data in variant call format (.vcf). Together the SNPfisher tools provide a valuable and simple resource for probing the genetic variation in commonly used zebrafish strains, facilitating use of zebrafish variation data for generating sequence-specific reagents, for distinguishing natural variation from mutation-causing defects in positional cloning projects, and for zebrafish genome-wide association studies.

164. Gene-Breaking Transposon Mutant Resource. *Mark D. Urban, Rhianna M. Harm, Tammy M. Greenwood, Melissa S. McNulty, Kelly S. Predmore-Rogers, Kari L. Williams,* ***Karl J. Clark****, Stephen C. Ekker.* Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN.
Even with advances in custom genome engineering, GBT lines are of high value in discovery of the function of understudied genes, provide expression data through mRFP, permit rapid genotyping for husbandry and comparative studies, and permit conditional rescue through excision using Cre recombinase. Gene-break transposon vectors, like pGBT-RP2.1, result in fluorescently tagged, highly mutagenic, and reversible mutant alleles of zebrafish genes. In these lines, a red fluorescent protein (mRFP) is encoded by an artificial 3' exon, whose expression occurs as a result of a translational fusion to an endogenous locus. These GBT insertions are highly mutagenic with over 97% loss of the normal wild-type transcript in homozygous mutants. Insertions into genes with known alleles have phenocopied the previously identified mutations. In total, we observe phenotypes during the first 5 days of development in about 7% of the GBT lines screened to date, which is comparable to other zebrafish mutant datasets. We will provide an update on our GBT production and distribution of data through zfishbook.org- a central hub for molecular, expression and mutational information about GBT lines in production by researchers from around the globe. As of March 1, 2014 we have catalogued 768 mutagenic lines. Of these lines, 618 have a complete image summary consisting of dorsal, sagittal, and ventral images at both 2 and 4 dpf of the mRFP expression pattern. 508 of the lines have been cryopreserved, with 411 of these lines already shared with ZIRC. In collaboration with the Human Human Genome Research Institute, we have tagged 274 lines to genetic loci. We have shipped 389 zebrafish lines to 39 laboratories at 36 separate institutions.

165. Homologous Recombination (HR) based genome editing using the CRISPR-Cas mechanism in zebrafish. ***Thomas Clements****, Farid Abu-Shamat, Jordan Rothfeld, Wesley Chou, Daniel Wagner.* Biochemistry and Cell Biology, Rice University, Houston, TX.
Genome editing has reached numerous milestones in mice, but the translation of this technology to the zebrafish, Danio rerio, remains difficult. Zebrafish are a powerful vertebrate model organism because of their large clutch size, rapid development, and transparent embryos. This has led zebrafish to become a premier forward genetics model through such techniques as ENU and insertional mutagenesis. However, zebrafish reverse genetics is still somewhat cumbersome. One emerging technique in this field is centered on inducing double-strand breaks (DSBs) in DNA with site specific endonucleases resulting in deletions and insertions (in/dels) from errors in non-homologous end joining (NHEJ). While creating site-specific in/dels is a powerful mutagenesis method, homologous recombination (HR) based genome engineering would lead the zebrafish genetic model to be on par with the mouse. Our goal is to be able to efficiently engineer the same type of knock in reporters and conditional alleles that are available in the mouse. Unfortunately, zebrafish lack the embryonic stem cell technology that makes selection for these types of rare recombination events efficient. Thus, we must promote HR to increase the rate of gene conversion using engineered targeting vectors by increasing HR and/or suppressing NHEJ. To increase the number of targets we can survey, we are utilizing the recently described CRISPR-Cas endonuclease complex to induce DSBs at precise locations and promoting HR over NHEJ at the break site to efficiently edit the zebrafish genome. In/dels have been observed in all of our targets either by observation of pigment loss or by utilizing an enrichment assay. We have also observed a 6.25% germline transmission of in/dels in one of our target genes. Currently, we are working to optimize our methods to increase the efficiency of targeting and the percentage of germline transmission. Once we have done so, we plan to take this technique and link it to factors to promote HR or inhibit NHEJ. The the ability to carefully control the amount of mRNA introduced in the embryo and the transient expression of mRNAs will allow us to identify a set of conditions that will allow efficient gene targeting in the zebrafish.

166. AMAGEN: a fish transgenesis platform for biomedical research. ***Noemie de Croze****, J Edouard, Z Radev, D. Barbachou, M. Couseau, E. Dijoux, C. Haye, B. Maurice, P Lafaux, L. Legendre, F. Sohm.* UMS AMAGEN CNRS-INRA, Institut de Neurobiology A. Fessard Bat. 32, Gif sur Yvette, France.
Identification of new genes involved in disease and development by recent large screen analysis now require validation through in vivo

functional analysis. Transgenesis is a keystone technique in both in vivo promoter analysis and reverse genetic. Since 2007, the French platform AMAGEN promotes Zebrafish (Danio rerio) but also Medaka (Oryzias latipes) as a genetic model organism for biomedical research. AMAGEN provides a range of services to both academic and private researcher related to transgenesis. AMAGEN is able to produce custom transgenic zebrafish lines as well as provide wildtype zebrafish lines. AMAGEN is partner of the tefor infrastructure, which provide integrated service from vector production to transgenic lines production and develop new phenotyping technic. In this frame, AMAGEN provides custom knock out lines and is optimizing methods using TALE nuclease to produce targeted knock-in fish lines. Here, we will present the different services provided to the french and international research community by AMAGEN.

167. Analysis of RNAseq datasets from an infectious disease model using GeneTiles. ***Jan de Sonneville***[1], *Wouter Veneman*[2], *Kees-Jan van der Kolk*[1], *Anita Ordas*[2], *Zaid Al-Ars*[3], *Annemarie Meijer*[2], *Herman Spaink*[2]. 1) Life Science Methods BV, Leiden, Netherlands; 2) Institute of Biology, Leiden University, Leiden, Netherlands; 3) Computer Engineering, Delft University of Technology, Delft, Netherlands.
Visual inspection of RNA sequencing (RNAseq) data in 1D visualization programs, where reads are mapped on a line representing the genome, requires constant zooming, scrolling and offers almost no overview of the results. As manual inspection using such visualization software is difficult and a time consuming task, this creates a gap between data collection and analysis, traditionally closed by bioinformaticians. We improved the visualization and browsing of RNAseq data in an online user-friendly platform called GeneTiles. Open source alignment and statistics programs required to map raw illumina data-files are integrated in this platform and the whole data analysis pipeline is kept open for scientific inspection and reproducibility. Differential expression of genes in a chromosome is shown as a 2D array of tiles. The tile color and intensity are a measure of the significance of the number of reads of experiment versus control. When a tile is selected, the gene is loaded underneath, scaled to fit the width of the screen. In a schematic view all introns are shrunk to a fixed short length to visualize the aligned reads in a graph above the exons. We used GeneTiles on a large dataset of bacterial infection in a zebrafish model. We have previously compared different bacterial infection systems by combining micro-array and RNAseq data, however, in this study we were able to identify much faster and more confidently a set of infection markers upon infection with *Staphylococcus epidermidis* and *Mycobacterium marinum*. We have shown the applicability of this approach also to published RNAseq datasets of other organisms by comparing our data with a published mammalian infection study. In addition we have used the DEXSeq module in GeneTiles to quickly identify genes, such as glucagon A, that are differentially spliced under infection conditions.

168. Expanding the annotated gene set for the zebrafish community. ***Sarah Donaldson***, *Gavin Laird, David Lloyd, Kerstin Howe, John Collins, Jennifer Harrow.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
The new release of the zebrafish genome GRCz10 is due this year and manual annotation is provided by the Human and Vertebrate Analysis and Annotation (HAVANA) group. The annotation is released quarterly via the Vertebrate Genome Annotation (VEGA) database (vega.sanger.ac.uk). A new Havana Update track in VEGA is now available which publishes any changes to annotation every two weeks. The manual annotation is also merged with the automatic ensembl gene build to produce a high quality reference gene set in Ensembl (www.ensembl.org). Our annotation is performed in close collaboration with the Zebrafish Information Network (zfin.org), which enables us to provide an accurate, dynamic and distinct resource for the zebrafish community. Manual annotation is traditionally based on EST, cDNA and/or protein homologies which is limited in zebrafish compared to human and mouse. We make increasing use of a wide range of transcriptome data, including in-house RNAseq data from a range of developmental stages and tissues, and transcriptome data supplied by collaborators or imported from publications. Additionally, we import Ribosome Profiling, poly(A)-position profiling (3P-seq) and CAGE TSS data and a host of comparative analysis, allowing us to produce a comprehensive gene set. This comprehensive set of genes includes numerous loci not previously described elsewhere, including non-coding and novel coding genes. The lncRNA gene set has expanded considerably recently, with over 700 lncRNAs present in VEGA. The incorporation of the new data sets has led to significant improvements in the quality, quantity and comprehensiveness of the annotated zebrafish genes in VEGA. External requests for manual annotation are welcome, as are suggestions for, or offers of, prospective new data sets or collaborations. Please contact zfish-help@sanger.ac.uk to discuss these.

169. Establishment of Gene Targeting Method for Zebrafish Using the Engineered Endonuclease. ***Zhangji Dong***, *Wenshuang Jia, Xiaohua Dong, Shasha Cao, Qingshun Zhao.* Model Animal Research Center, MOE Key Laboratory of Model Animal for Disease Study, Nanjing University, Nanjing 210061, China,.
Gene targeting is a genetic technique that uses homologous recombination to change an endogenous gene by deleting a gene, removing exons, inserting a gene, or introducing point mutations. Though knockout zebrafish have been routinely generated in research labs by using engineered endonucleases including ZFN (zinc finger nuclease), TALEN (transcription activator-like effector nucleases) and CRISPR/Cas9 to induce indel mutations in an endogenous gene of zebrafish genome, gene targeting method for zebrafish remains impractical for the researchers to use because of extremely low efficiency. To improve the efficiency of gene targeting in zebrafish embryos, we explored the effects of different factors on the rate of homologous recombination occurring nearby the double strand break created by engineered endonucleases in an endogenous gene of zebrafish embryonic cells. By optimizing the factors that affecting homologous recombination in germ cells, we successfully obtained heritable gene targeting zebrafish carrying perfect loxP in introns of a gene, which will enable to generate conditional knockout alleles of the gene in zebrafish. In summary, our results provided a practical method of gene targeting for zebrafish using the engineered endonuclease. Keywords: zebrafish, gene targeting, CRISPR/Cas9, ZFN, TALEN.

170. Adult Phenotypic Screen of Molecularly-Defined Mutant Genes. ***Ricardo Fuentes***[1], *Amy Kugath*[1], *Gui Hu*[1], *Tim Cooper*[2], *Joanna Fried*[1], *Catherine Scahill*[4], *Chike Nwaezeapu*[1], *Jean Cooper*[2], *Luyuan Pan*[3], *Cecilia Moens*[3], *Keith Cheng*[2], *Elisabeth M. Busch-Nentwich*[4], *Derek L. Stemple*[4], *Shannon Fisher*[1], *Mary C. Mullins*[1]. 1) University of Pennsylvania, Philadelphia, PA, USA; 2) Penn State College of Medicine, Hershey, PA, USA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 4) Wellcome Trust Sanger Institute,

Cambridge, UK.
Large-scale forward genetic screens have been highly successful in the zebrafish to identify gene functions in development or other biological processes. However, these screens are limited due to the time-consuming effort required to positionally clone the mutated genes. A more rapid and cost-efficient method of assessing all gene functions in a vertebrate is a broad-based phenotypic analysis of mutant genes generated by reverse genetic strategies. The Sanger Institute is currently engaged in the identification of a large set of molecularly-defined mutations in protein-coding genes in zebrafish by whole exome sequencing. Analysis of a subset of these mutant genes indicates that 90% do not have an apparent function during embryonic development. We postulate that a significant fraction of these genes are required during late larval, juvenile, or adult stages. To test for post-embryonic gene functions, we are taking advantage of the reverse genetic mutant resources in the zebrafish to screen at the adult stage 1200 molecularly-defined mutant genes that are embryonic viable. By using morphological, histological, radiographic, molecular and cellular approaches, we have identified numerous maternal-effect, male sterile, adult morphological, and late larval lethal mutant genes. We will present our results and analysis of the mutant phenotypes. Our goal is to generate a resource of late larval to adult gene-phenotype information to provide to the zebrafish community. Our phenotyping screen provides an unbiased analysis of gene function beyond embryonic stages. Furthermore, it allows us to discover novel regulatory factors acting during larval and adult stages, as well as maternal regulators of embryogenesis, which will generate important molecular-genetic entry points for future in-depth studies of gene function.

171. Using Nile red, monodansylpentane and Bodipy to visualize vascular lipid deposition in zebrafish larvae fed on a high cholesterol diet. ***Marcel den Hoed***, *Manoj K Bandaru, Erik Ingelsson.* Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden.
Zebrafish larvae develop hypercholesterolemia and vascular lipid deposition when fed on a high cholesterol diet, making it a promising model system for early-stage human atherosclerosis. So far, the fluorophore cholesteryl ester boron-dipyrromethene (Bodipy) has been used to stain vascular lipid deposition. Unfortunately the excitation-emission spectrum of Bodipy overlaps with EGFP, thereby complicating the use of Bodipy with many existing transgenic zebrafish lines. The aim of the present study was to examine the possibility to stain vascular lipid deposition in live zebrafish larvae using Nile red and monodansylpentane, and compare staining patterns across dyes. We fed zebrafish larvae on a high cholesterol diet from day 5 to 15 post-fertilization. Bodipy was administered on days 14 and 15 by enrichment of the diet at 60 mg/g. Nile red and monodansylpentane were administered by soaking larvae for 60 min in 0.5 mg/ml Nile red, or for 45 min in 25 mM monodansylpentane. Given their excitation-emission spectrum, we were able to co-stain vascular lipid deposition using Bodipy and monodansylpentane (n = 13), and using Nile red and monodansylpentane (n = 10). Optical sections of the caudal vein (mean length 395 ± 18 mm) were acquired using a Leica SP5 confocal microscope. The high cholesterol diet resulted in a variable response in vascular lipid deposition, ranging from 0 to 168 droplets across individual larvae. Staining by Nile red and monodansylpentane overlapped completely in all larvae. Staining by monodansylpentane and Bodipy overlapped completely for 6 of 13 larvae. The remaining larvae showed either more (12 ± 1, n = 2) or fewer (-9 ± 5, n = 5) lipid droplets with monodansylpentane as compared with Bodipy. In conclusion, Nile red and monodansylpentane can both be used to stain vascular lipid deposition in live zebrafish larvae. Incomplete overlap of staining by monodansylpentane and Bodipy likely reflects the presence of cholesterol-free lipid droplets and binding site competition.

172. Bone mineralization in the regenerating zebrafish fin. *Anthony Recidoro*[1], *Werner Kaminsky*[2], ***Ronald Kwon***[1]. 1) Orthopaedics and Sports Medicine, University of Washington, Seattle, WA; 2) Department of Chemistry, University of Washington, Seattle, WA.
The defining feature of osteoporosis is low bone mineral density (BMD), an integrated measure of bone mass and mineralization that is highly associated with fracture risk. By virtue of its capacity to recapitulate the major phases of human osteogenesis, the regenerating zebrafish tail fin provides a compelling model for BMD therapeutic discovery. However, a major hurdle to such efforts is the lack of quantitative modalities for mineralization analysis. Thus, our goals were twofold: 1) to assess the correspondence between Rotopol microscopy (a quantitative form of birefringence imaging), microCT, and Raman spectroscopy (complimentary modalities for mineralization analysis in mammals); and 2) to determine whether Botulinum toxin (BTx), a paralytic neurotoxin broadly associated with osteogenic dysfunction in mammals, impairs mineralization during fin regeneration. For Rotopol imaging, we utilized a custom polarized light microscope to sequentially acquire images under a stepwise rotating polarizer. This enabled birefringence to be decoupled from transmittance and orientation, enabling quantitative analysis. In regenerated bone rays, BMD (via microCT) and birefringence (via Rotopol imaging) were highly correlated (R2=0.88), with correspondence occurring over the entire bone ray except near joints in non-regenerated tissue (due to birefringence from the fibrous inter-joint ligaments). In BTx-treated fish, Raman analysis revealed a 30 percent reduction in the v1 phosphate/proline ratio. Further, Rotopol imaging revealed a significant decrease in birefringence ($p<0.05$ relative to saline controls), suggesting BTx impaired bone mineral accumulation. Together, these studies directly advance the regenerating fin as a model of bone mineralization by establishing sensitive and quantitative assays for this process. More broadly, by establishing correspondence between light- and X-ray-based imaging modalities, these studies take a significant step toward integrating high-content fluorescent microscopy with quantitative mineralization imaging for systems-based investigations of BMD accrual.

173. Zebrafish Transgenic Line *huORFZ* As an Effective Living Bioindicator for Detecting Environmental Toxicants. *Hung-Chieh Lee*[1], ***Po-Nien Lu***[1,2], *Hui-Lan Huang*[1,2], *Chien Chu*[3,4], *Hong-Ping Li*[3], *Huai-Jen Tsai*[1]. 1) Institute of Molecular and Cell, National Taiwan University, Taipei, Taiwan; 2) Liver Disease Prevention & Treatment Research Foundation, Taipei, Taiwan; 3) Taiwan Agricultural Chemicals and Toxic Substances Research Institute Council of Agriculture, Executive Yuan, Taichung, Taiwan; 4) Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan.
Animal models are invaluable for monitoring the extent of pollution in the aquatic environment. In this study, we demonstrated that *huORFZ*, a novel transgenic zebrafish line that harbors a human upstream open reading frame of the *chop* gene fused with GFP reporter, as a bioindicator for monitoring environmental pollutants and stress-related cellular processes. Under normal condition, no GFP signal could

be detected on *huORFZ*. When treated with hazardous chemicals, including heavy metals and endocrine-disrupting chemicals near their sublethal concentrations (LC50), *huORFZ* embryos exhibited different tissue-specific GFP expression patterns. Copper (Cu^{2+}), cadmium (Cd^{2+}) and Chlorpyrifos were selected for further investigate. Cu^{2+} triggered GFP responses in skin and muscle, whereas Cd^{2+} triggered GFP responses in skin, olfactory epithelium and pronephric ducts. Moreover, fluorescence intensity exhibited by *huORFZ* embryos was dose-dependent. After chemical treatments, the surviving embryos were returned to normal condition, and survival rates, apoptosis signals, returned to normal levels with no significant morphological defects. Also, GFP signals decreased along with recovery, suggesting that GFP signaling of *huORFZ* embryos likely reflected the overall physiological condition of the individual. To examine the performance of the *huORFZ* line under real-world conditions, we placed *huORFZ* embryos in different river water samples. We found that the *huORFZ* embryos correctly detected the presence of various kinds of pollutants. Based on these findings, we concluded that such uORFchop-based system can be integrated into a first-line water alarm system monitoring the discharge of hazardous pollutants. *At the time of submitting this abstract, the study has been accepted by PLOS One and is in press.

174. *sim1a* Mediates Renal Progenitor Patterning During Zebrafish Nephrogenesis. ***Christina N. Cheng***, *Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
During zebrafish development, an embryonic kidney known as the pronephros arises from renal progenitors in the intermediate mesoderm. Initial patterning of the renal progenitor field is reliant on retinoic acid (RA) signaling, which regulates the formation of rostral and caudal domains. Once the pronephros is established, it consists of two nephrons that have proximal and distal segments similar to mammals including humans: the proximal convoluted tubule (PCT), proximal straight tubule (PST), distal early (DE), Corpuscle of Stannius (CS), distal late (DL), and a duct. To date, the mechanisms that establish nephron segmentation patterning remain poorly understood. Using whole mount *in situ* hybridization (WISH) to profile renal progenitor gene expression, we discovered that the transcription factor *single minded homologue 1 (sim1a)* marks the caudal domain. *sim1a* expression was dynamic during nephrogenesis, being maintained in both proximal tubule segments before becoming restricted to the CS. Since these findings suggested that *sim1a* might contribute to segment patterning and CS formation, we performed functional analysis of *sim1a* using morpholinos. *sim1a* deficiency caused an expansion of the PCT, which was minimally functional as indicated by a dextran-FITC uptake assay, and an abrogation of the PST and CS populations. However, the domains of both the DE and DL segments remained unchanged. Our results suggest that *sim1a* is necessary to pattern the PST and CS, and that *sim1a* may negotiate the PCT/PST boundary, possibly by inhibiting PCT fate choice. Furthermore, an overall proximalization of nephron tubule identity was observed in *sim1a* morphants following the addition of exogenous RA. Also, when *sim1a* morphants were chemically treated with diaminobenzaldehyde (DEAB), a reversible inhibitor of RA synthesizing enzymes, the distal segments became expanded at the expense of the proximal regions. Taken together, our study reveals novel roles for *sim1a* during nephrogenesis and suggests that this gene functions downstream of RA. These findings provide useful insights into the genetic pathways that direct nephron development, and may have implications for understanding the causes of renal birth defects in humans.

175. *ELABELA* in Heart Development. ***Serene C Chng***, *Lena Ho, Bruno Reversade.* Institute of Medical Biology, Human Genetics and Embryology Laboratory, A*STAR, Singapore 138648, Singapore.
We have recently discovered and characterized a highly conserved gene, *ELABELA* (*ELA*), encoding a hormone of 32 amino acids. Utilizing zinc-finger-nuclease-mediated gene inactivation in zebrafish, we generated a series of *ela* mutants. *ela* null embryos displayed impaired endoderm development as marked by reduced *gata5* and *sox17* expression. *ela* null embryos have little or no heart tissue, phenocopying the loss of the *apelin receptor (aplnr)*. We show that Aplnr is Ela's cognate receptor and together, form an essential signalling axis which crosstalks with Tgfb for early cardiogenesis.

176. Hoxb5b Controls the Midline Convergence of the Foregut Endoderm. ***Gokhan Dalgin***, *Victoria Prince.* Organismal Biology and Anatomy, University of Chicago, Chicago, IL.
The zebrafish gut tube gives rise to internal organs and forms from a bilateral monolayer of endoderm cells. Coordinated movement of endoderm cells towards the midline is crucial for generating internal organs in the correct locations. During gastrulation stages the bilateral endodermal cell movements require cues from endoderm, mesoderm and also from the extra-embryonic yolk syncytial layer. Cell tracing analysis suggests that during subsequent segmentation stages, the mediolateral and anteroposterior location of the endoderm cells determine their cell fate choices. However, the signals guiding endoderm cell migration towards the midline are not completely understood. Here we show that the Hoxb5b transcription factor is required for the midline convergence of foregut endoderm. Using a morpholino knockdown approach we have established that down-regulation of hoxb5b throughout the embryo causes a bifurcated gut tube phenotype. Interestingly, we have found that pancreatic cells are specified but fail to differentiate properly in the bifurcated endoderm. To better characterize endoderm cell migration we have used time-lapse microscopy of embryos carrying the endoderm-specific reporter (Tg:sox17GFP). We have found that in hoxb5b-depleted embryos midline convergence of the endoderm halts at 14 hpf, a stage when hoxb5b is expressed in both the endoderm and surrounding mesoderm. To determine in which germ layer Hoxb5b is required for endoderm migration, we are now performing a series of cell transplantation experiments to allow germ layer specific down-regulation of Hoxb5 function. Identification of the germ layer in which Hoxb5b functions to influence endoderm cell movements will be crucial to establishing a full understanding of the migration mechanisms.

177. Thyroid development in zebrafish lacking *TAZ*. *A. Pappalardo*[1,2,3], *I. Porreca*[3,4], ***E. De Felice***[3], *L. Caputi*[3], *S. Schulte-Merker*[5], *M. Zannini*[1], *P. Sordino*[3,6]. 1) Institute of Experimental Endocrinology and Oncology 'G. Salvatore' - CNR, 80131 Naples, Italy; 2) IRCCS Fondazione Stella Maris, 56018 Calambrone, Pisa, Italy; 3) Stazione Zoologica Anton Dohrn, 80121 Naples, Italy; 4) IRGS, Biogem, 83031 Ariano Irpino, Avellino, Italy; 5) Hubrecht Institute-KNAW and University Medical Centre, 3584 CT Utrecht, The Netherlands; 6) Institute for Mediterranean and Forestal Systems (ISAFOM-CNR), Catania, Italy.

TAZ (Transcriptional co-Activator with PDZ binding motif) is a signal-responsive transcriptional co-regulator implicated in several biological functions. Herein, we have used zebrafish to study the role of *TAZ* as a candidate regulator of thyroidal development. Zebrafish thyroid reveals high degree of conservation at the cellular and molecular levels with mammals. Similar to vertebrates, the functional unit of the fish thyroid is the follicle. Follicular cells of the zebrafish thyroid express the transcription factors *nk2.1a, pax2a, pax8* and *hhex*, necessary for thyroid differentiation as well as the proteins involved in thyroid-hormone synthesis such as *slc5a5*; they ultimately produce thyroglobulin, accumulate iodine and synthesize T4. *TAZ* mRNA co-localizes with different territories of the zebrafish embryo, including the developing thyroid. The abolition of the TAZ protein through morpholino microinjection did not alter the expression of thyroid markers. Remarkably, RT-qPCR revealed that blocking *TAZ* induced a marked enhancement of *Tg* and *slc5a5* mRNA expression. We showed a marked decrease in the mean number and size of thyroid follicles, a defect not previously described in *TAZ* morphant zebrafish. Also, loss of *TAZ* results in abnormal morphogenesis/remodeling of cranial blood vessel causing ectopic follicles. Concerning MSC development, *TAZ* abrogation induced complete absence of cartilage and bone tissues, as supported by gene expression analysis. Interestingly, loss-of-function embryos carried excess adipoblasts that failed to form mature fat cells, in support of the hypothesis that *TAZ* is implicated in late stages of adipogenesis. These findings reveal that *TAZ* is not required for the early differentiation of the thyroid and are the first to suggest that *TAZ* has a role in size control of the endocrine gland.

178. Identification of Retinoic Acid Signaling Components Essential for Renal Progenitor Development in the Zebrafish. ***Eric K. Donahue***, *Shahram J. Poureetezadi, Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
Zebrafish have emerged as a valuable model to study the kidney due to their high degree of genetic conservation with humans. Retinoic acid (RA) signaling during zebrafish development is essential to pattern renal progenitors into discrete proximo-distal nephron cell type lineages. In a wild type zebrafish, RA acts to induce proximal segments from the intermediate mesoderm. Upon addition of exogenous RA, proximal segments expand at the cost of distal fates. Conversely, inhibition of RA production using diethylaminobenzaldehyde (DEAB) eliminates proximal segment formation and expands the distal segments. Previously, we found that a retinoic acid receptor alpha (RARa) chemical agonist AM-580 was shown to have similar effects as RA to promote proximal fates, while the RARa antagonist R041-5232 inhibited proximal fates and expanded distal fates, suggesting a role for RARa during renal progenitor development. However, further analysis of RA receptors during nephrogenesis has not been performed. Chemical genetic screening is a relatively new technique that allows for the phenotypic assessment of small molecules to determine if they can alter signaling pathways affecting development, organogenesis, and/or other biological processes *in vivo*. Zebrafish embryos can be easily drug treated by adding small molecules to the embryo media, making them an ideal model for chemical genetics. Through an ongoing chemical genetic screen, we have identified a growing category of compounds that alter proximo-distal nephron segmentation that includes a number of RA receptor agonists and antagonists. Among them are compounds that regulate retinoid X receptor (RXR) activity, thus suggesting a role for RXRs in addition to RARa for proper renal development. Future studies will delineate the time window when alterations in RA receptor activity affect nephrogenesis, and identification of the specific genes that are involved. Elucidating these aspects of RA signaling will provide useful information to better understand the mechanisms of this pathway during nephron development.

179. Lineage specification of multipotent progenitors in the liver and pancreas. ***P. Duc Dong***, *Danhua Zhang, Keith Gates, Joseph Lancman.* Genetic Diseases, Sanford-Burnham Med. Rsh Inst, La Jolla, CA.
As a source of multipotent progenitors, ducts of the liver and pancreas are thought to play a key role in the development and disease of these organs. Despite considerable progress by a multitude of investigations into the genetic mechanisms governing morphogenesis and differentiation of the hepatopancreas ductal system, it has been difficult to demonstrate which particular genes are required to initially establish the entire ductal compartment of the pancreas and liver. We determined that a common signaling factor expressed in endoderm derived cells within the liver and pancreas is necessary to induce duct fate in both these organs. We further uncovered the transcriptional effectors downstream of this signaling pathway required for pancreas ductal lineage specification. In the absence of the pancreatic ductal compartment, neogenic endocrine cells fail to form. Surprisingly inhibition of acinar fate can restore endocrine development, indicating that the duct lineage is not essential for endocrine fate. Our findings are consistent with a model in which activation of this signaling pathway leads to transcriptional reprogramming of a subset of acinar and hepatocyte precursors to adopt duct fate instead. Contrary to the current paradigm suggesting a central role for ductal progenitors, our studies indicates that the ductal compartment of the liver and pancreas is not required for other organ cell types nor is it critical for the growth and morphogenesis of the liver and pancreas. These discoveries resolve longstanding questions of how the vertebrate intra hepatopancreatic duct compartment is specified and the role of this compartment in pancreas and liver development.

180. Notochord vacuoles are essential for body axis elongation and proper spine morphogenesis. ***Kathryn L. Ellis***[1], *Jennifer Bagwell*[1], *Jamie Mankiewicz*[1], *Xiaoyan Ge*[2], *Jason Wiler*[3], *Nicholas Katsanis*[3], *Didier Y. Stainier*[4], *Michel Bagnat*[1]. 1) Dept. of Cell Biology, Duke University, Durham, NC; 2) Dept. of Biochemistry, University of California, San Francisco, CA; 3) Center for Human Disease Modeling, Department of Cell Biology, Duke University Medical Center, Durham, NC; 4) Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany.
The notochord plays critical structural roles during vertebrate development. At the center of the vertebrate notochord is a large fluid-filled organelle, the notochord vacuole. We have recently shown that zebrafish notochord vacuoles are specialized lysosome-related organelles that require late endosomal trafficking regulated by the vacuole-specific Rab32a and H+-ATPase-dependent acidification. We establish that notochord vacuoles are required for body axis elongation during embryonic development and identify a novel role for notochord vacuoles in spine morphogenesis. In order to examine the role of notochord vacuoles in spine formation, we are taking a forward genetic approach to identify novel molecular players in notochord vacuole formation and spine morphogenesis. We isolated several “chubby” mutants in our lab with defects in notochord vacuole biogenesis that display a shortened body axis. Characterization and mapping of these mutants will

elucidate novel molecular players involved in notochord vacuole biogenesis. Using live imaging and genetic manipulations, we are visualizing the relationship between the notochord vacuoles and the developing spine in vivo. We are analyzing dwarf mutants, identified in the Stainier lab, that display a shortened body axis and scoliosis of the spine. dwarf mutant embryos show disrupted notochord vacuoles in an otherwise straight notochord. Using live imaging we show that it is not until vertebrae maturation that the spine in dwarf mutants becomes kinked as bone grows around the notochord. These studies suggest that lack of a hydrostatic scaffold for bone deposition in fish with disrupted notochord vacuoles leads to aberrant spine morphogenesis. We propose that fully inflated notochord vacuoles are necessary to evenly distribute the compressive force of growing vertebrae during spine development.

181. Interspecific cell transplants to elucidate evolutionary changes in pigment pattern formation in two species of danios. ***Emily J Bain***, *David M Parichy*. Biology, University of Washington, Seattle, WA.
Understanding how evolutionary changes in development lead to the diversity of adult form requires knowing how differences at the molecular level lead to variation in differentiation and morphogenesis. Pigment patterns among Danio fishes offer an interesting system in which to study post-embryonic development and evolution as externally visible patterns are critical for animal behavior and ecology. Fish are particularly tractable for such analyses because their patterns comprise cells containing pigment granules that act as natural markers of the cell's location. Stripes of the zebrafish, *Danio rerio*, consist of stripes of black melanophores separated by light interstripes of yellow xanthophores and iridescent iridophores. In the closely related species, *Danio albolineatus*, the same pigment cell types are intermingled leading to a more uniform pattern. Previous studies have shown that the timing and differentiation of pigment cells, as well as interactions between them, are essential to the *D. rerio* pattern. However, these same mechanisms in *D. albolineatus* remain largely unexplored. We are testing if evolutionary differences between these two species are intrinsic to pigment cells, or if stripe loss in *D. albolineatus* is due to changes in the environment in which pigment cells reside. To distinguish between these possibilities, we are transplanting both single pigment cell types and combinations of pigment cell types from *D. rerio* into *D. albolineatus*, as well as *D. albolineatus* pigment cells into *D. rerio* mutants deficient for particular pigment cell classes. Based on the outcome of these transplants, we will target gene discovery efforts and rescue experiments to identify changes in molecular mechanisms underlying stripe loss in *D. albolineatus*.

182. Analysis of *duplicated doppelgängers* and *paired paralogs* for Mapping the Ancestral Whole Genome Duplication of Teleost Fish. ***Danielle Beekman***, *Albert Erives.* Dept. of Biology, University of Iowa, Iowa City, IA, 52242-1324.
A whole genome duplication (WGD) event played a foundational role in shaping the evolution of teleost fish. Now, with the recent availability of multiple sequenced genome assemblies from this clade and closely-related outgroups, it is possible to study the nature of this WGD and assess the evolutionary fates of duplicated genes in greater detail. Starting with Ensembl's BioMart database query system and its orthology-calling pipeline, we identified ~500 mapped, duplicated gene pairs that are shared by zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) but are present as single copy genes in the spotted gar (*Lepisosteus oculatus*), a non-teleost ray-finned fish. By analyzing the evolution of the teleost karyotype *via* these ~1,000 duplicated genes, we find we can assign membership of each gene to one of two ancestral genome copies. To enable teleost-wide comparative genomic analyses, we indexed these gene pairs according to their location in the ancestral syntenic blocks. All duplicated genes in the same syntenic block receive either the suffix "*-dd*" or "*-pp*", which stand for "*duplicated doppelgänger*" and "*paired paralog*", respectively. This robust WGD-related gene paralogy naming system facilitates discussion of the ancestral teleost genome, while allowing the WGD-related gene duplicates to be easily distinguished from the many haphazard paralogy naming conventions as well as lineage-specific paralogy. Using the details gleaned from our syntenic paralogy maps, we speculate on the nature of the teleost WGD, including the possibility that it was an allo-tetraploidization event (*i.e.*, a hybridization of two divergent actinopterygian genomes). For example, we find that many of the genes involved in the Notch signaling pathway (*N1*, *rbpj*, *furin*) and transcription-related complexes (*ep300*, *sin3a*) are maintained as duplicates across teleosts. Because duplicated developmental loci harboring multiple transcriptional enhancers are frequently subject to regulatory sub-functionalization, an updated genomic map of the teleost WGD may lead to new insights magnifying the power of zebrafish genetics. In addition, our study has the potential to lead to novel, practical insights into both WGD events and karyotype evolution.

183. Evolution of excitation-contraction coupling: evidence for divergence of fast and slow twitch modes in teleost fish. ***Jens P C Franck***, *Sara V Good, Tim Pellisier, Sergey Yegorov.* Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada.
Excitation contraction (EC) coupling describes the relationship between the depolarization of the muscle membrane and the subsequent contraction of the muscle cell. In skeletal muscle cells the depolarization of the muscle membrane triggers a conformational change in the L-type calcium channel ($Ca_V1.1$). In skeletal muscle, the $Ca_V1.1$ directly interacts and mechanically gates the intracellular ryanodine receptor (RyR) channel to release calcium from the intracellular stores of the sarcoplasmic reticulum, a mechanism termed depolarization induced calcium release (DICR). In contrast, the L-type channel in cardiac muscle ($Ca_V1.2$) opens in response to the depolarization signal and the extracellular calcium subsequently acts as a ligand to gate open the intracellular RyR channels, a mechanism termed calcium induced calcium release (CICR). The DICR mode of calcium release is believed to be a vertebrate innovation. We previously described fiber type-specific expression of RyR1 paralogs in slow twitch (ryr1a) and fast twitch (ryr1b) muscle fibers in fish. More recently it has been reported that the alpha1S subunits (a_{1S}) of the multimeric $Ca_V1.1$ channel are also duplicated and expressed discretely in the slow twitch (a_{1S}-a) and fast twitch (a_{1S}-b) muscles of zebrafish. The II-III loop of the a_{1s} subunit and the divergent region 1 (DR1) of RyR1 have been implicated as the domains responsible for the mechanical coupling process. Utilizing selection analyses we show that amino acids within the a_{1S} II-III loop and RyR1 DR1 regions are under positive selection. Additionally, the nodes leading to the fast twitch-specific genes (ryr1b and a_{1S}-b) show a greater extent of positive selection than the nodes leading to the slow twitch-specific genes (ryr1a and a_{1S}-a). Multiple sequence alignments reveal evidence for the fixation of functionally divergent amino acids in the RyR1 DR1 and a_{1S} II-III loop domains. We discuss the implications of these results and the potential for the coevolution of the functional domains responsible for the DICR mode of EC coupling in teleost fish.

184. Formation of the first tooth is necessary and sufficient for dental row development in zebrafish. ***Yann Gibert***[1], *Eric Samarut*[2], *Megan Ellis*[1], *Vincent Laudet*[2]. 1) Deakin School of Medicine, Geelong, Australia; 2) Institut de Genomique Fonctionelle de Lyon, France.
Zebrafish unlike other teleost fish lost anterior teeth during evolution but retain pharyngeal dentition on the posterior most pharyngeal arch while most teleosts bear teeth on the anterior pharyngeal arches. Development of the first forming tooth in zebrafish requires fibroblast growth factor (FGF) and retinoic acid (RA) signalling. However inductive signals required for the development of the subsequent teeth, 3V1 and 5V1 of the dental row remain unknown. Induction of 3 and 5V1 occurs in a stereotype pattern and we hypothesized that the first tooth, 4V1, may play an active role in the induction of the dental row. We chemically manipulate the FGF and RA pathways in vivo and showed that they are necessary for the induction of the later teeth. Interestingly depletion of the first forming tooth prevents the development of the dental row and tooth formation post 4V1 ablation will always start again by de novo formation of a single first tooth, making 4V1 formation mandatory for dental row formation. Moreover exogenous RA signalling during early neural crest specification induces the formation of anterior ectopic single tooth formation. Once formed these ectopic anterior teeth are each able on their own to induce subsequent teeth of the denture: 3 and 5V1. Our results demonstrate that 4V1 is both necessary and sufficient to induce denture formation, which explains why 4V1 is under high selection constraints and why ablation of this tooth will reprogram tooth pattern induction with one single tooth formed first. Moreover our results also show that zebrafish possess the necessary information to form anterior teeth in the pharyngeal region where RA signaling is normally absent.

185. Different Levels of Notch Signalling are Needed for Arterial Specification and Haemogenic Endothelial Cell Induction in the Dorsal Aorta. ***Mohammad H Al Khamees***, *Sunny Modhara, Markus Owen, Chris Moore, Martin Gering.* School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH UK.
During zebrafish embryogenesis, angioblasts located below the notochord are induced to express arterial genes, like *efnb2a*, as they begin to form the dorsal aorta (DA). Several hours later, endothelial cells in the ventral wall of the DA (vDA) turn haemogenic, express haematopoietic transcription factors like Runx1 and cMyb, and give rise to definitive blood cells. Loss of Notch signalling results in the abrogation of arterial specification and definitive haematopoiesis. As Notch ligand and receptor expression are maintained in the DA throughout this period, it remained unclear whether the induction of haemogenic ECs (hECs) required Notch only for arterial specification or whether Notch was in addition specifically involved in hEC induction. Notch gain-of-function suggested that high levels of Notch are sufficient to induce ectopic arterial specification and hEC formation. We previously noticed that *mibta52b* mutants and *rbpja/b* morphants consistently lost both arterial and hEC marker expression, whereas embryos treated with the g-secretase inhibitor DAPM lost *runx1* and *cmyb* expression, but retained arterial *efnb2a* expression. Here, we use Notch reporter lines to monitor the level of Notch activity during DA development and hEC induction in wild-type and Notch-depleted embryos. We present confocal data and quantitative RT-PCR data to show that Notch reporter protein and mRNA levels increase from the time of arterial specification to the time of hEC induction. We also show in quantitative RT-PCR experiments that Notch reporter gene expression is eliminated in *mib* mutants and *rbpja/b* morphants, while DAPM-treated embryos retain some Notch activity. Altogether, these data suggest that low levels of Notch signalling are sufficient for arterial gene expression, while high levels of Notch signalling are required for hEC induction.

186. Coiled-coil domain containing protein 103 regulates myeloid development. ***Sarah A Beckman***, *Joshua Waxman.* Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.
Myelopoiesis is the process of generating all types of myeloid cells, including macrophages and granulocytes. Myeloid cells have many important roles including immune surveillance and tissue remodeling. Abnormal myeloid function can result in diseases such as leukemias and myeloproliferative disorders including idiopathic myelofibrosis. Therefore, understanding regulators of myelopoiesis and myeloid function will lead to novel therapies for myeloid-related disorders. The coiled-coil domain containing protein 103 (Ccdc103) was recently identified as being mutated in smh, a zebrafish model of primary ciliary dyskinesia (PCD), as well as being mutated in humans with PCD. Ccdc103 was found to have a role in dynein arm assembly and cilia motility. We initially found that Ccdc103 is expressed in the anterior lateral plate mesoderm (ALMP) at 17s, where it was not previously reported. Further confirming it's possible role in myelopoiesis, we found that Ccdc103 is downstream of the master hematopoietic regulators Scl and Lmo2 and myeloid regulator Pu.1. To determine if Ccdc103 is expressed in human myeloid cells, we investigated the expression of Ccdc103 in myeloid leukemia cell lines and cord blood and found expression in the majority of the lines tested. Together, these data suggest a previously unknown role for Ccdc103 in myelopoeisis. To further characterize the role of Ccdc103 in myelopoiesis, we performed knock-down and overexpression studies. Knocking down Ccdc103 using morpholinos (MOs) that phenocopy the smh phenotype cause a decrease in neutrophil and macrophage numbers at 1dpf. We were able to rescue the myeloid cell depletion in Ccdc103 morphants by co-injection with ccdc103 mRNA, while over-expressing ccdc103 mRNA results in an increase in both neutrophil and macrophage numbers at 1dpf. Together, these data implicate a role for Ccdc103 in myeloid cell production. Interestingly, Ccdc103 morphants also displayed abnormal progenitor cell migration at 17s, with some Pu.1 positive cells migrating anteriorly instead of over the yolk sac. We conclude that Ccdc103 may have a previously unrecognized role in myeloid production and migration and may be an exciting new target for myeloid-related disorders.

187. The Role of the Spliceosome in Regulating DNA Methylation in Hematopoiesis. ***Rosannah C. Cameron***, *Teresa V. Bowman.* Molecular and Developmental Biology, Albert Einstein College of Medicine, Bronx, NY.
The spliceosome is an essential piece of cellular machinery responsible for regulating gene expression and increasing transcript diversity through alternative splicing. Spliceosomal mutations have the potential to be profound and affect numerous transcripts. Mutations in components of the spliceosome are common in myelodysplastic syndrome (MDS), which is a disease of ineffective hematopoiesis resulting from hematopoietic stem cell dysfunction. The insertional zebrafish mutant *hi3394* contains a mutation in the spliceosomal component *splicing factor 3b, subunit 1* (*sf3b1*). Using *in situ* hybridization for the hematopoietic stem and progenitor cell (HPSC) marker *runx1*, we have found that *hi3394* mutants have greatly diminished HSPC formation. Additionally, we found that these mutants also have lower

amounts of mature red blood cells. These data show that similar to humans, zebrafish with mutations in spliceosomal factors also have blood defects. In addition to mutations in spliceosomal genes, many MDS patients also have mutations in epigenetic machinery suggesting a relationship between the spliceosome and epigenetic modifications. The concept that DNA methylation can affect splicing through the generation of alternative transcripts has been well documented, however the idea that transcription could regulate DNA methylation is relatively new. To investigate the relationship between the epigenetic modifications and the spliceosome, we are currently performing whole genome bisulfite and transcriptome sequencing of HSPCs isolated from wild type and mutant embryos. We will compare DNA methylation and gene expression changes between mutant and wild types. This analysis will allow us to determine which genes are differentially methylated or alternatively spliced and the connection between the two traits in mutant HSPCs. This research will provide new insight into the underlying molecular mechanisms that cause MDS as well as generating valuable information about hematopoiesis and gene regulation in general.

188. Splicing and epigenetic control of hematopoietic stem cells. ***Adriana De La Garza****, Teresa Bowman.* Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY.
Defects in hematopoietic stem cells (HSCs) lead to hematological diseases such as myelodysplastic syndromes (MDS) and leukemia. Recurrent mutations in spliceosomal components were recently identified in these diseases, but how they might lead to disease is unclear. Using zebrafish genetics, we plan to dissect the molecular nature of these mutations and delineate the path to hematologic dysfunction when splicing is altered. Additionally, as epigenetic factors are also commonly mutated, we will explore how they interact with the spliceosome during HSC formation. Zebrafish mutants in U2 snRNP components, *sf3a3*^{hi1950} and *sf3b1*^{hi3394}, have diminished HSC formation and vascular defects by 28 hours post fertilization (hpf). Using these mutants, we will perform a genetic interaction screen by knocking down chromatin factors using morpholinos and looking for changes in HSC formation in embryos heterozygous for U2 snRNP mutations. *chd1* is a chromatin modifier that has been shown to interact with U2 snRNP in human cells and to recruit it to chromatin. *In vivo*, we found that combination of *chd1* knockdown in an *sf3a3* heterozygous background causes a further decrease of HSC levels in comparison to chd1 knockdown or *sf3a3* heterozygosity alone. Classes of epigenetic factors being screened include DNA methyltransferases, histone deacetylases and histone methyltransferases. Once we identify interacting candidates we will further characterize the mechanism of interaction and impact on genome-wide splicing and epigenetic modification. Our findings will give further insight into hematologic pathologies and their treatment.

189. Mammalian TRIM27 is functionally equivalent to zebrafish Bloodthirsty in erythroid differentiation —. ***H. William Detrich***[1]*, Sandra Parker*[1]*, Paul Kingsley*[2]*, James Palis*[2]. 1) Marine and Environmental Sciences, Northeastern University Marine Science Center, Nahant, MA; 2) School of Medicine and Dentistry University of Rochester Medical Center, Rochester, NY.
The zebrafish *bloodthirsty* (*bty*) gene encodes a protein that belongs to the tripartite motif (TRIM) family. We have shown previously that embryos injected with antisense morpholinos (MOs) targeted to the 5' end of the *bty* mRNA fail to produce erythrocytes. Bioinformatic analysis indicates that *bty* is orthologous or paralogous to one of two mammalian genes, *TRIM27* or *TRIM39*. To determine whether one, both, or none are functionally equivalent to *bty*, we have tested the ability of synthetic murine *Trim27* and *Trim39* mRNAs to rescue erythrocyte production in zebrafish *bty* morphants. Here we show that *Trim27* mRNA rescues erythropoiesis in zebrafish embryos when co-injected with *bty* MOs, whereas *Trim39* mRNA does not. Using a mouse erythroid-lineage gene microarray, we find that *Trim27* transcript levels are significantly higher both in primitive and in definitive erythroid precursors compared to *Trim39* transcripts. Furthermore, *Trim27* mRNA levels decline rapidly as primitive and definitive erythroid maturation proceeds, consistent with two potential roles for the Bty/Trim27 protein: 1) targeting transcriptional repressor(s) of proerythroblast differentiation for ubiquitin-mediated proteasomal degradation; or 2) repressing the expression of these inhibitors. *Trim39* transcript levels, by contrast, remain low throughout both waves of erythroid maturation. Finally, we show by *in situ* hybridization that *Trim27* transcripts are expressed in emerging yolk sac blood islands of mouse embryos (E8.5) but are downregulated in circulating primitive erythrocytes (E12.5). Both *Trim 27* and *Trim39* are expressed at high levels in fetal liver (E12.5). Taken together, our functional and gene expression studies indicate that *Trim27* is the probable mammalian ortholog or paralog of zebrafish *bty* and support an important role for *Trim27* in erythroid cell maturation. Supported by NSF grants OPP-0944517 and PLR-1247510 (HWD) and by NIH grant DK079361 (JP).

190. Investigating the role of the sclerotome and the downstream targets of non-canonical Wnt signaling in hematopoietic stem cell development. ***Nicole Glenn****, Clair Kelley, Chaithanyarani Parupalli, Wilson Clements.* Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN.
Hematopoietic stem cells (HSCs) provide all blood and immune cells during life and comprise the therapeutic component of bone marrow transplants. Finding immune-compatible donors remains a problem. One solution is to generate HSCs in vitro. Understanding how the embryonic HSC specification niche is formed and what signals are presented there is one means of identifying additional HSC specification signals. Recently non-canonical signaling through Wnt16 was found to be required for HSC specification through a series of downstream relay signals. The final signal(s) remain to be determined, but do not appear to involve any known HSC specification pathways. The sclerotome compartment of the somite appears to be involved in relaying the Wnt16 signal to HSCs. We have taken a two-pronged approach to distinguishing between the possibilities that Wnt16 regulates sclerotome gene expression or morphogenetic behavior. First we are examining the signal transduction downstream of Wnt16. Preliminary studies indicate that the atypical Wnt receptors Ror1 and/or Ryk are candidate Wnt16 receptors, and that Tak1, a kinase involved in Ror signal transduction is not involved. As Ror family receptors and Ryk have been found to form higher order complexes, we are examining the possibility that Frizzled-family receptors contribute to differential Ror and Ryk signaling outputs, as well as the possibility that there are competing Wnt ligands. Second, we are generating transgenic animals with sclerotome-specific gene expression, in order to perform live imaging studies of sclerotome behavior, non-surgically ablate sclerotome, sort and transcriptionally interrogate sclerotomal cell populations, and perturb sclerotomal signal transduction

using transgenically expressed dominant negative and active proteins. We have defined promoter elements from pax1 and twist2 capable of driving sclerotome-specific expression. These studies will define non-canonical Wnt signaling downstream of atypical receptors and identify the molecular factors and cellular behaviors that regulate HSC-specification niche formation.

191. Fam49ab Is a Novel Regulator of Lymphopoiesis in Zebrafish. ***Roman Li***, *Julien Bertrand.* PATIM, University of Geneva, Geneva, Switzerland.
Hematopoietic stem cells (HSCs) are pluripotent and self-renewing cells that replenish all blood and immune system lineages throughout the entire life of an individual. Deregulation of haematopoiesis can lead to leukaemia, immunodeficiency and autoimmune disease. Gene therapy of patient-derived iPS cells followed by *in vitro* differentiation into HSCs is an attractive potential therapeutic strategy to treat those diseases. However *de novo* generation of HSCs is currently not possible, indicating that our understanding of this process is not complete. We therefore set out to find new regulators of haematopoiesis by analysing the transcriptome of HSCs in zebrafish. We report that *fam49ab* is a gene of unknown function that is highly expressed in HSCs. Overexpression of *fam49ab* inhibits lymphopoiesis while having no effect on erythroid or myeloid fates. Preliminary results show that this hematopoietic phenotype is cell-autonomous, as the thymic epithelium is not affected. We are now investigating the relationship of *fam49ab* to the PTEN pathway, as this pathway is required for lymphopoiesis. Our first results suggest that *fam49ab* could physically interact with some components of the PTEN pathway in an inhibitory fashion. Altogether, these data characterise a new protein of a previously unknown function and its involvement in HSCs differentiation.

192. Sexual Maturation of Zebrafish Within 46 Days Post-Fertilization. ***Y. Aoyama***[1], *N. Moriya*[2], *S. Tanaka*[2], *A. Inazuki*[2], *T. Taniguchi*[2], *H. Hosokawa*[2,3], *S. Maegawa*[2,3]. 1) Department of Human Coexistence, Kyoto University, Kyoto, Japan; 2) Division of Cognitive and Information Sciences, Kyoto University, Kyoto, Japan; 3) Department of Intelligence Science and Technology, Kyoto University, Kyoto, Japan.
Rearing larvae with high growth rate and survival rate is one of the challenging issues for all zebrafish researchers. Saltwater rotifers (*Brachionus plicatilis*) have been reported as a good food for newly hatched larval fish based on high growth rate and survival rate. Larval fish, however, have to tolerate high salt conditions in the method because saltwater rotifers require the high salt conditions. We here show a new rearing method by using freshwater rotifers (*Brachionus calyciflorus*), which achieves high larval growth and survival without the high salt conditions. Moreover, our method accelerates sexual maturation of zebrafish from 3 months to 46 days. Three independent rearing experiments revealed that the larvae fed with freshwater rotifers showed 3.5 times higher growth rate than the larvae fed with processed diets from 5 to 12 days post-fertilization (dpf). In addition, 90.0% (81/90) of the larvae fed with freshwater rotifers were survived at 30 dpf. Finally, viable embryos were obtained from natural breeding with the male and female AB fish at 46 dpf. The fastest sexual maturation was observed at 38 dpf in the case of a AB/TU male fish. Using this rearing method, three-generation genetic analyses in zebrafish can be completed within five months. Our method improves the status of the zebrafish as a model organism, and will facilitate our understanding of developmental processes.

193. A Novel Approach to Understanding the Physical Processes behind High Throughput Cryopreservation of Zebrafish Sperm. ***Richard C. Clark***, *Elisabeth M. Busch-Nentwich, Ross N. W. Kettleborough, Christopher M. Dooley, Catherine Scahill, Neha Wali, Samantha Carruthers, Amanda Hall, Zsofia Pusztai, Richard Gibbons, Derek L. Stemple.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
With the goal of the Zebrafish Mutation Project (ZMP) to generate knock out models of every protein coding gene in the genome and distribute these to the International and European Zebrafish Resource Centers (ZIRC and EZRC), there is a need to gain further insight and develop the ZMP high throughput cryopreservation and IVF protocols. Cryopreservation has long been the key process in the storage and distribution of a wide variety of important genetic materials. However, robust cryopreservation of Zebrafish sperm is difficult to reproduce partly due to a lack of understanding of the actual freezing process. Better knowledge and understanding of this will provide insight into improved freezing conditions and help translate these parameters into an optimal and reproducible cryopreservation protocol. In addition to robust freezing protocols, it is also important to develop reliable methods maximising in vitro fertilisation (IVF) success to ensure finite material is used efficiently during the regeneration of these unique Zebrafish lines. Here is described the Wellcome Trust Sanger Institute's (WTSI) high throughput cryopreservation method with new insight into the physical processes that occur during freezing, their relevant time points and how subtle changes in the protocol, reagents and consumables affect the physical freezing process. Also demonstrated is the accompanying IVF protocol with recent results for the resurrection of new family lines from ZMP cryopreserved sperm.

194. Myonecrotic and fluorescent granulomas in *Danio rerio* infected by the microsporidian pathogen *Pseudoloma neurophilia*. *Kylie West*[1], *Rodney Miles*[2,3], *Michael L. Kent*[4], ***J. Kimble Frazer***[1]. 1) Pediatric Hematology-Oncology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) ARUP Laboratories, Salt Lake City, UT; 4) Departments of Microbiology and Biomedical Sciences, Oregon State University, Corvallis, OR.
Zebrafish (*Danio rerio*) are useful human disease models. Like other metazoans, *D. rerio* can be infected by pathogens. One potential threat are microsporidia, intracellular fungus-like parasites. Fish ingest microsporidian spores, which germinate and spread throughout the host. Microsporidia can infect the central nervous system (CNS), skeletal muscle, and other sites, impairing breeding and causing morbidity or even mortality. Zebrafish infected by microsporidia may develop abnormal spine contour due to CNS infiltration, altered pigmentation, wasting, or other physical signs. Such signs may suggest infection, which can be confirmed by histology or tests for microsporidian nucleic acids. In our *D. rerio* colony, we discovered many fish with auto-fluorescent nodules in flank musculature. Histologic examination showed these nodules were granulomas containing inflammatory cells, spores, and degenerating muscle tissue. When zebrafish with lesions were housed with other fish, multiple healthy fish acquired fluorescent nodules, indicating a transmissible condition. Further histologic analysis

showed CNS lesions, a classic pathologic feature in fish infected by the microsporidian *Pseudoloma neurophilia*. PCR testing of muscle and CNS tissue from nodule-bearing fish detected *P. neurophilia* DNA in both sites, but PCR for *Pleistophora hyphessobryconis*—another microsporidian that infects *D. rerio* skeletal muscle—was negative. Collectively, our findings reveal an unusual, previously-unreported type of *P. neurophilia* infection that is characterized by auto-fluorescent muscle granulomas. Since lesions fluoresce, detecting *P. neurophilia*-induced granulomas is feasible in living fish. This means at-risk colonies can easily be monitored for this type of infection, and microscopy-based screens for susceptibility or resistance to these granulomas are possible. Moreover, our results indicate the native zebrafish pathogen *P. neurophilia* is a novel granulomatous disease model.

195. A Novel Short Rogranulin of Tilapia Modulates Innate Immunity in Transgenic Zebrafish Model. ***H. Gong***[1,2], *C. Tang*[1], *S. Wu*[1], *W. Lin*[1]. 1) Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan; 2) Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan.
A novel short progranulin (PGRN) gene encoding 199-a.a. secretory protein composed of 20 a.a. signal peptide and 179 a.a. short PGRN with two granulin (GRN) units was identified from both Mozambique and Nile tilapia and was named as PGRN-S199. The OmPGRN-S199 was abundantly expressed in tilapia immune-related tissues including head kidney, spleen and intestine. Both gram-negative Vibrio vulnificus and gram-positive Streptococcus iniae bacterial infection can induce PGRN-S199 mRNA expression in Mozambique tilapia. The weak and strong PGRN-S199 transgenic zebrafish lines driven by muscle-specific promoter had been established, respectively in order to study the function and molecular action of PGRN-S199 on the regulation of fish innate immunity. Upon Vibrio vulnificus infection, the survival rate was enhanced in both weak and strong OmPGRN-S199 transgenic zebrafish lines. In the absence of bacterial injection, innate immune-related genes including IL-8, TNFa, IL-6, NF-kB1, Rel-A, c-Rel, Rel-B, Cox-2a, Cox-2b etc., were suppressed in the OmPGRN-S199 transgenic zebrafish compared to wild type zebrafish. In contrast, the innate immune-related genes especially pro-inflammatory genes such as IL-8, IL-1b, TNFa, IL-6, IL-22, COX-2a and Mx in OmPGRN-S199 transgenic zebrafish were highly activated at 3 hpi and 6 hpi following challenge with Vibrio vulnificus. It suggests OmPGRN-S199 should be processed into GRNs to induce inflammation response to defense bacterial infection in transgenic zebrafish treated with Vibrio. Furthermore, the OmPGRN-S199 can enhance the innate immune response to elevate pro-inflammatory gene expression and advance the time to 3 hpi in transgenic zebrafish to against bacterial infection. In conclusion, the novel tilapia short PGRN-S199 can regulate innate immune-related genes to exert anti-inflammation activity in zebrafish without bacterial pathogen infection, whereas PGRN-S199 will be converted into GRN peptides to strengthen innate immune response to enhance survival of zebrafish against bacterial pathogen.

196. Lipidomic analysis during embryonic development. ***Daniel Fraher***[1], *Andrew Sanigorski*[1], *Natalie Mellett*[2], *Peter Meikle*[2], *Andrew Sinclair*[1], *Yann Gibert*[1]. 1) Deakin School of Medicine, Australia; 2) Baker IDI Heart and Diabetes Institute, Australia.
Metabolism has a fundamental role in biological processes including embryonic development and tissue homeostasis. However to date, little is known about the lipid requirements, profile, abundance and metabolism during vertebrate embryogenesis. During development, the zebrafish embryo can be considered as a “closed system”, since the embryo relies on its yolk sac reservoir for growth. This aspect allows for a model that is uninfluenced by external nutrition. To understand what lipids are present during embryogenesis, we performed full lipidomic analyses of the yolk sac and the embryo body at different crucial stages of zebrafish embryogenesis: 1 cell-stage, 24 hours post fertilization (hpf), 48 hpf, 72 hpf and 120 hpf (end of embryogenesis). In this study, we analysed 375 lipid species that were grouped into 24 categories. Interestingly, all lipid species identified in the yolk sac were also found in the embryo body while the reciprocal is not true. Our analyses revealed that the yolk sac is a reservoir of triglyceride. Of the main lipid classes, the yolk sac contained 1.8x more total lipids, 4.3x more of both TAG and PS, 4.8x more LPC, 2.9x more PI, 1.9x more cholesterol, 2.1x more CE, and 1.7x more PC than the embryo body at 48 hpf. In contrast, the yolk sac contained only 0.34x as much PS, and 0.16x as much as the total DHC (dihexosylceramide) in the embryo body. To investigate the role of PPARg in lipid availability during development, we analysed the lipid changes when this gene was pharmacologically blocked during zebrafish embryogenesis. Lipidomic analyses of zebrafish embryos allow an unprecedented detailing of lipid dynamics during vertebrate embryogenesis. These analyses are useful to study the roles of lipids within disease contexts, in genetically manipulated models, or for pharmacological studies.

197. Adipogenesis is regulated by additive actions of the Endocannabinoid System and Retinoic Acid Pathways. ***Daniel Fraher***, *Megan Ellis, Alister Ward, Ken Walder, Yann Gibert.* Deakin School of Medicine, Geelong, Australia.
Obesity affects nearly 500 million people worldwide and is becoming an epidemic as the increases in unhealthy eating habits and weight gain promote the risk of cardiovascular diseases and type 2 diabetes. Recent studies have implicated that the endocannabinoid system (ECS) and the retinoic acid (RA) pathway are involved in the development of adipose. To elucidate these roles, we activated and inhibited the ECS in developing zebrafish by chemical treatment with the ECS agonist Win 55212-2 and antagonist Rimonabant. Similarly, zebrafish were treated with exogenous RA and the RA synthesis inhibitor DEAB. Fish exposed to ECS and RA activation showed an increase in lipid deposition (detected with Oil-red-O staining), while blocking the activity of these pathways reduced lipid deposition. Furthermore, manipulation of these pathways affected the expression of key genes involved in adipocyte differentiation and fat metabolism, including C/ebpa, lpl, and fabp11a in both zebrafish and in mature mammalian adipocyte cells. We have also shown that the two pathways have an additive effect. Combined treatment with sub-optimal chemical doses from both pathways was sufficient to affect changes in lipid deposition and lpl expression during zebrafish embryogenesis. Furthermore, we have treated embryos with RA receptor and cannabinoid receptor selective inhibitors and activators to further define lipid regulation. Also, whole-embryo lipidomic analysis revealed that manipulation of the two pathways caused specific changes in the lipid profile of the developing embryo. These results provide detailed roles for the ECS and RA pathway in the regulation of lipid metabolism and adipogenesis and create the potential for manipulation of the pathways to treat obesity.

198. Understanding the role of miR-375 in early pancreatic islet development. ***Ankit Gupta***[1], *Dana DeSantis*[1], *Scot Wolfe*[1,2]. 1) Program in Gene Function and Expression, UMass Medical School, Worcester, MA; 2) Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, MA.
One of the voids in understanding the gene regulatory network that drives the development of b-cells is the role of miRNAs in this process. miR-375 is the most abundant miRNA present in b-cells and is also present in pancreatic endocrine progenitor cells. Deletion of miR-375 in mice results in lower b-cell mass and hyperglycemia. Although multiple target genes of miR-375 have been identified, its role in early pancreatic development remains unexplored. We employed zinc finger nucleases (ZFNs) to create zebrafish loss-of-function mutants of miR-375. Since miR-375 is present in two copies (miR-375-1 and miR-375-2) in the zebrafish genome, we created carriers transmitting 7 bp deletions at both miR-375-1 and miR-375-2 loci. These deletions cause loss of function in a miRNA-sensor assay. Similar to morphants, zebrafish embryos carrying mutant alleles of miR-375-1 and miR-375-2 display morphological defects in the pancreatic islet starting at ~60 hpf. These morphological defects are accompanied by a significant reduction in the b-cell number and an increase in the glucose levels at 6 dpf confirming a role of miR-375 in early pancreatic development. In order to identify spatial and temporal expression pattern of miR-375 during development, we created transgenic animals where the Gal4FF transgene is inserted at the miR-375-2 locus. The resulting Gal4FF expression mimicked the miR-375 expression and labeled endocrine cells in a variety of tissues throughout development. We could detect miR-375 expression in developing pancreatic islet as early as ~17 hpf, which is before distinct populations of a- and b-cells have been specified. These tools will allow us to identify and validate regulatory targets of miR-375 and understand the role of miR-375 and its targets in a- and b- cell specification.

199. Investigating the Role of Rab25 in Maintaining EVL Integrity during Zebrafish Epiboly. ***Molly A Allen***, *Ashley Bruce.* Cell and Systems Biology, University of Toronto, Toronto, Canada.
Morphogenesis during embryonic development, when cell movements eventually give rise to the tissues and organs of animals, can result in severe patterning defects if not properly regulated. We study the earliest morphogenetic event during zebrafish development, epiboly, which is defined as the thinning and spreading of a multilayered cell sheet. In a coordinated manner the three layers of the early zebrafish embryo, the deep cells, the enveloping layer (EVL) and the yolk syncytial layer (YSL) all undergo epiboly to thin and spread the blastoderm over the yolk cell to close the blastopore. Zygotic genes that may be important for epiboly initiation were identified through RNA-Sequencing by comparing the transcriptome of wild type embryos at sphere and dome stages. One gene up-regulated at dome stage and predominantly expressed in the EVL was rab25, which encodes a small GTPase that has been identified as a key component in cellular trafficking through apical recycling in polarized epithelial cells. Integrity of the EVL is essential for normal epiboly, thus we were interested in exploring the potential role of Rab25, since it has been associated with epithelial cell integrity in a number of systems, yet its function has never been examined in zebrafish. Preliminary experiments using over-expression, dominant-negative constructs, and antisense morpholino oligonucleotide approaches suggest that Rab25 plays a role in maintaining the integrity of the EVL revealed by an apparent reduction in EVL tension at the margin and abnormal cell morphology. Ongoing experiments aim to characterize these defects in more detail by examining cellular trafficking of apical markers, tight junction components, and adherens junction components in the EVL.

200. The Organization and Dynamics of Yolk Cytoplasmic Microtubule Networks during Zebrafish Epiboly. *Zhonghui Fei*, ***Koeun Bae***, *Ashley Bruce.* Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada.
Morphogenesis describes the coordinated spatial distribution and rearrangement of cells in an organism. To study morphogenesis, we focus on zebrafish epiboly - the coordinated spreading of the blastoderm, from its starting position on top of the large yolk cell, over the yolk surface. Work from several groups has implicated microtubule networks in the yolk cytoplasm (YCL) in this process, but their exact role is unclear. Using live imaging, we examined various aspects of the network organization and dynamic during epiboly. The movements of microtubule plus end associated protein EB3 was examined by injection of RNA encoding EB3 fused to GFP. EB3-GFP movements revealed constant nucleation of new yolk microtubules during epiboly and extensive unidirectional microtubule growth in the vegetal direction in the YCL from high to 60% epiboly stage, after which point the growth stops in the YCL, but nucleation continues. We have generated a fluorescently labeled tubulin transgenic line that allows maternal expression of fluorescent tubulin. We are currently performing fluorescent recovery after photobleaching (FRAP) experiments on the tubulin transgenic embryos to test the hypothesis that the YCL microtubule networks are composed of short tubulin polymers similar to the organization found in axons. Furthermore, using fluorescently labeled doublecortin (microtubule associated protein) transgenic embryos, we demonstrated that the networks can move both animally and vegetally with high variability. In addition, our data suggest that yolk syncytial nuclei pulling in epiboly may be facilitated by newly formed microtubules, rather than by existing YCL networks. Together, these results provide insights to the organization and dynamics of the YCL microtubule networks, and in turn, their potential function during epiboly.

201. Semicircular Canal Formation in Zebrafish. ***Sarah Baxendale***, *Esther C Maier, Rachael Lawrence, Tanya T Whitfield.* Bateson Centre, Department of Biomedical Science, University of Sheffield, Sheffield, South Yorkshire, United Kingdom.
The inner ear has the dual function of detecting sound and motion, enabling us to hear and to maintain balance. During development the inner ear transforms from a simple ball of epithelial cells into a complex labyrinth to detect sound, gravity, linear acceleration and rotational movement. This process of epithelial remodelling to generate form is a fundamental biological process and one that is beautifully demonstrated in the ear. Morphogenesis of the semicircular canal system in the zebrafish embryo begins when projections of tissue, driven by extracellular matrix production, move into the lumen of the otic vesicle. Here, they fuse to form pillars of epithelium that span the vesicle lumen and provide structural support for the developing ear. We are aiming to understand semicircular canal formation and function at the molecular, cellular and behavioural level. At the molecular level, we have identified genes by mutant analysis, expression pattern and homology to other species. We are studying different mutant lines where specific canal ducts fail to form correctly. In the *gpr126* mutant (*lauscher*), the projections that form the pillars over-express a number of ECM components, including *versican* genes. The projections

become dysmorphic, overgrow, and fail to fuse to make pillars, resulting in defects in formation of all three canals. The *otx1* gene is required for formation of the ventral canal pillar and specification of the lateral semicircular canal, whereas in the *cloudy* mutant, there are specific defects in formation of the dorsal epithelium of the anterior and posterior canal ducts. To follow cellular movements during formation of the canal system we are using confocal and light sheet microscopy imaging in real time in transgenic zebrafish embryos expressing GFP throughout the otic epithelium. Canal formation is accompanied by dramatic changes in cell shape and adhesion and we are comparing cell dynamics in wild-type and mutant embryos through to adult fish. Malformation of the semicircular canals leads to balance defects in zebrafish and we are using automated movement tracking in adult homozygous fish to characterize the different movement behaviours associated with different canal defects.

202. Perdurance of maternal Fascin1 masks essential roles for Fascin1 in regulating cranial neural crest migration. ***Elena Boer***[1], *Elizabeth Howell*[1], *Thomas Schilling*[2], *Cicely Jette*[1], *Rodney Stewart*[1]. 1) Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Developmental and Cell Biology, University of California Irvine, Irvine, CA.
Maternal gene products deposited in the oocyte are required to regulate the earliest stages of embryonic development prior to zygotic genome activation. Previous studies have shown maternally expressed genes and miRNAs play essential roles in early developmental processes, including egg activation, cleavage, axis specification, and gastrulation. In contrast, roles for maternal products in later stages of vertebrate embryonic development, such as organogenesis, remain largely uncharacterized. Fascin1 is an actin-binding protein that bundles actin fibers to generate filopodia in migrating cells. We generated TALEN-induced null mutations in zebrafish *fscn1* and found that zygotic *fscn1* is dispensable for viability, as *fscn1* null homozygous mutants are viable and fertile. In contrast, embryos devoid of both maternal and zygotic *fscn1* (*MZ fscn1*) displayed defects in development of the neural crest-derived craniofacial skeleton and peripheral nervous system. Analysis of Fscn1 protein levels in zygotic *fscn1* mutants revealed that maternally supplied Fscn1 endures for at least 7 days, masking an early requirement for zygotic *fscn1* in migrating cranial neural crest cells. In addition, *MZ fscn1* mutants provide a sensitized genetic background to identify modifier genes that act together with *fscn1* to control directional migration of cranial neural crest cells. We will present our findings on *fscn1*-dependent pathways in collective neural crest migration, the novel role of maternal Fascin1 in craniofacial development, and bioinformatic analysis of maternal genes to identify additional genes that behave like *fscn1* to regulate later embryonic stages.

203. Characterizing normal choroid fissure formation and the underlying roles of cell adhesion. ***Chase D Bryan***, *Kristen M Kwan.* Human Genetics, University of Utah, Salt Lake City, UT.
Vertebrate visual function is dependent on the proper formation of the embryonic optic cup which establishes the basic structure of the eye, containing neural retina and retinal pigment epithelium enwrapping the lens. In zebrafish, this occurs between 12-24 hours post fertilization (hpf). One particular structure of interest that arises during optic cup formation is the choroid fissure, a transient, yet crucial structure necessary for visual function. Its development generates the ventral channel through which vasculature enters the eye and retinal axons exit. While the choroid fissure forms during optic cup morphogenesis, it subsequently fuses along its length (24-72 hpf). Failure of choroid fissure formation or fusion results in a structural defect known as coloboma, which is associated with visual impairment in humans. To understand the defects that cause colobomata, we must first understand how the structure normally forms. Using 4-dimensional timelapse imaging and cell tracking methods we previously developed, we determined the cell movements underlying the formation of the choroid fissure during optic cup formation. We find that the choroid fissure margins arise from a single population of cells that moves out of the brain and into the optic vesicle during evagination, and splits apart to form the nasal and temporal margins of the choroid fissure during optic cup invagination. It is unclear what drives this split; we hypothesize that cell adhesion must be modulated in order to separate and define the two distinct margins. Cell adhesion, both to the extracellular matrix as well as between cells, is known to play a role in choroid fissure development: zebrafish with mutations in cell adhesion components display colobomata. Until now, it was unclear whether colobomata were due to defects in choroid fissure formation during early optic cup morphogenesis, or fusion during later development. Here, we show that the colobomata seen in *laminin-a1*$^{-/-}$ and *cadherin2*$^{-/-}$ mutant embryos result from early defects in choroid fissure formation. Moving forward, we aim to determine the specific cellular events disrupted in each mutant underlying choroid fissure defects, including cell movements, cell death, and mitosis.

204. Expression Analysis of *sox9b:eGFP* Reporter Constructs in Transgenic Zebrafish With and Without 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Exposure. ***Felipe R. Burns***[1], *Kong M. Xiong*[2], *Joseph C. Gawdzik*[1,3], *Andrew Sentkowski*[3], *Richard E. Peterson*[3], *Warren Heideman*[1,3]. 1) Molecular and Environmental Toxicology Center; 2) Department of Biomolecular Chemistry; 3) School of Pharmacy, University of Wisconsin, Madison, WI.
Zebrafish *SRY-box containing gene 9b* (*sox9b*) is necessary for proper morphogenesis of craniofacial cartilages and is repressed by the aryl hydrocarbon receptor (AHR) agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The *sox9b* locus resides in a region devoid of other genes. It is unknown how the proximal promoter of *sox9b* uses long-range enhancers nor how TCDD represses *sox9b* transcript abundance. We aimed to find elements 5' of *sox9b* needed to recapitulate *sox9b* expression and responsiveness to TCDD in developing zebrafish. Reporter constructs were made containing various *sox9b* promoter lengths fused to eGFP. Transient and transgenic expression data was gathered on five constructs using epi-fluorescence microscopy. *In silico* analysis of the *sox9b* locus reveled conserved non-coding regions containing putative cis-regulatory elements. Our results show transient and transgenic expression of the -2450/0*sox9b:eGFP* construct was able to recapitulate *sox9b* expression in many tissues at various times during embryonic and larval development. Zebrafish expressing eGFP under control of increasingly shorter lengths of the *sox9b* promoter showed transient expression in the same tissues the longest -2450/0 promoter reported in; however, the offspring exhibited more restricted expression patterns. Every construct recapitulated cardiac expression of *sox9b* under transient and transgenic conditions. RT-qPCR revealed that eGFP transcript was repressed by TCDD in tg(-2450/0*sox9b:eGFP*) zebrafish craniofacial cartilages to a lesser extent than *sox9b*. In conclusion, we have successfully captured a heart

specific enhancer in our shortest construct. Further, the longest construct captured an element recapitulating TCDD repression of *sox9b* in the craniofacial cartilages but lacked full *sox9b* behavior. Our results support alternate models of how the reporter constructs recapitulate *sox9b* expression by capturing a subset of *sox9b* enhancers while maintaining TCDD responsiveness.

205. Decreased collagen secretion causes reduced cranial facial development in zebrafish. ***Alicia Coughlin****, Caroline Lund, Po-nien Lu, Stephen Brannan, Michael Schoeneberger, Jennifer Liang*. University of Minnesota Duluth, Duluth, MN.
The cartilage and connective tissues of the face are formed largely by the cranial neural crest. *sec61al1* mutant zebrafish have defects in the translocation of newly synthesized proteins into the endoplasmic reticulum. We find that cranial facial development is reduced in these mutants as well. This reduction in cranial facial development is not caused by defects in the specification of cranial neural crest cells, but rather by reduced cartilage formation or collagen secretion. Cranial neural crest cells in *sec61al1* mutants migrated from the neural tube to the pharyngeal arches in a pattern that was indistinguishable from their WT siblings. Differences started to appear once the cranial neural cells reached the pharyngeal arches. Although developing cartilages were present, they were smaller than those of WT embryos and had altered morphology. For instance, Meckel's cartilage, which forms the lower jaw, was severely reduced or absent. The ceratohyal cartilage, which forms the ventral portion of the hyoid arch, was present but smaller. Further, Alcian blue staining throughout the head was lighter in *sec61al1* mutants, indicating collagen protein secreted into the extracellular matrix was reduced. These phenotypes worsened over time, with the structure of the cartilages becoming less distinct between 4 dpf and 5 dpf. Cranial neural crest cells never elongate Meckel's cartilage in *sec61al1* mutants. Thus, it is unlikely that the phenotype is simply a developmental delay. These data suggest that the abnormal formation of cartilage in the face of *sec61al1* mutants is due to reduced transport of collagen protein into the ER and thus reduced subsequent collagen secretion by cranial neural crest derived-chondrocytes.

206. Characterizing the Role of Junction Plakoglobin during Kupffer's Vesicle Morphogenesis. ***Agnik Dasgupta****, Jeffrey D. Amack.* Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY.
During early embryonic development, ciliated epithelial cells in a transient fluid-filled organ generate an asymmetric flow that is needed to establish the left-right (LR) body axis. Defects in the morphogenesis of this ciliated organ—called Kupffer's vesicle (KV) in zebrafish—disrupt LR patterning of the embryo. We are using KV as a model system to understand mechanisms underlying the development of this organ that directs LR asymmetry. Fate mapping studies have identified precursor cells, called dorsal forerunner cells (DFCs), which move along the leading edge of the dorsal side of the embryo and then undergo a mesenchymal to epithelial transition to form KV. At the start of the epithelialization process, multiple puncta that contain apical membrane and junction proteins form at cell-cell interfaces. DFCs then undergo rearrangements that bring these puncta, which we refer to as contact foci, into close proximity with one another to form the center of a rosette structure that gives rise to the nascent KV lumen. We are using time-lapse imaging of transgenic embryos to visualize the dynamics of DFCs during this process and identify genes that regulate DFC behaviors. Using a reverse genetic approach, we have found that Junction Plakoglobin (Jup) is involved in DFC epithelialization. Jup (also called g-catenin) is a close homolog of b-catenin that functions in cell-cell adhesion and cell signaling. Antisense depletion of Jup disrupts the arrangement of contact foci, formation of the rosette structure and subsequent KV morphogenesis. Consistent with KV defects, LR patterning is altered in Jup depleted embryos. We propose that Jup is instrumental in forming cell-cell adhesions that drive cohesion of contact foci during DFC epithelialization. Our results identify a new pathway that mediates morphogenesis of the KV organ, which is essential for establishing LR asymmetry of the embryo.

207. Spot pattern and size vary in original and regenerating caudal fins of zebrafish *leopard* mutants. ***Bethanie Borg****, Jennifer Liang, Students and Teaching Assistants in the Spring 2014 course Developmental Biology.* Department of Biology, University of Minnesota Duluth, Duluth, MN.
One of the fundamental questions in biology is how patterns are created. One relatively simple system where this is starting to be understood is the organization of pigment cells into patterns such as stripes and spots. One of the leading models for how pigment patterns are generated is through a reaction-diffusion system, based on equations developed by Alan Turing. In this system, a cell or group of cells expresses both a slowly-diffusing activator and a quickly-diffusing inhibitor. Pigmented stripes or spots would form at and around the cells where activator levels are high. In neighboring areas, the inhibitor levels would be higher than the activator, resulting in non-pigmented tissue. In a senior level undergraduate developmental biology course, students worked collaboratively to determine if stripe (WT fish) and spot (*leopard*/*connexin 41.8* mutants) formation in original and regenerating caudal fins is consistent with a reaction-diffusion system. One prediction from reaction-diffusion is that fish of the same size should have similar stripes or spots. While this was true for the striped fish, the caudal fins of *leopard* mutants had a wide range of patterns. For instance, in a comparable 9 mm^2 area of the fin, fish of the same age that were raised together could have between 0-15 spots. The spots also varied in shape from large, round ovals (largest with diameters of 1.7 and 0.65 mm, area of 0.9 mm^2) to small, elongated, narrow ovals (smallest with diameters of 0.58 and 0.14 mm, area of 0.05 mm^2). In regenerating fins, the reappearance of spots was delayed relative to stripes. Initial data indicates that many of the spots are not forming in the same places as the spots in the original fin. In contrast, when a spot was cut in half, the other half of the spot came back in the new tissue. Together, these data suggest that the reaction-diffusion system may not be the only mechanism involved in pigment patterning in the zebrafish fin.

208. The zebrafish adult epidermis derives entirely from the basal keratinocyte compartment. *Raymond Lee, PV Asharani,* ***Tom Carney***. IMCB, 31 Biopolis Drive, Proteos, Singapore.
The epidermis of terrestrial vertebrates is a stratified epithelium and forms an essential protective barrier. It is continually renewed, with dead corneocytes shed from the surface and replaced from a basal keratinocyte stem cell population. There is increasing employment of zebrafish genetics to analyse epidermis development and homeostasis, however there is a relative paucity of cellular markers and genetic reagents to label and manipulate the basal epidermal stem cell compartment. Furthermore, the architecture and ontogeny of the epidermis in

this system is incompletely described. In particular, it is unclear if adult zebrafish epidermis is derived entirely from the basal epidermal stem cell layer, as in the mouse, or if the most superficial keratinocyte layer is a remnant of the embryonic periderm. Here we show that the type I keratin, *krtt1c19e*, is a useful marker of the basal epidermal layer and identify a *krtt1c19e* promoter fragment able to drive strong and specific expression in this cell type. Use of this promoter to express an inducible Cre recombinase allowed permanent labelling of basal cells during embryogenesis, and demonstrated that these cells do indeed generate keratinocytes of all strata in the adult epidermis. Further deployment of the Cre-Lox system highlighted the transient nature of the embryonic periderm. We thus show that the epidermis of adult zebrafish, as in the mouse, is clonally derived from basal stem cells, further expanding the similarities of epidermal ontogeny across vertebrates, and highlights that a system of epidermal renewal from a basal stem cell compartment likely existed prior to adaption to land.

209. Investigating the Role of CCDC78 in Muscle Development and Disease. ***Ann E. Davidson***[1], *Xingli Li*[2], *James J. Dowling*[1,3]. 1) Genetics and Genome Biology, SickKids, Toronto, Ontario, Canada; 2) Neurology, University of Michigan, Ann Arbor, Michigan, USA; 3) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.
Congenital myopathies are a heterogeneous group of diseases that typically present in childhood with hypotonia and weakness and are commonly defined by characteristic changes observed on muscle biopsy. Currently, there are no known treatments or disease-modifying therapies. Therapy development is significantly hindered because approximately 40% of congenital myopathies are genetically unresolved. We recently identified a splice-acceptor variant of the coiled-coil domain containing gene (CCDC78) associated with an autosomal dominant congenital myopathy with distal predominant weakness. Muscle pathology presents central nuclei and core-like areas. In patients, CCDC78 is preferentially expressed in skeletal muscle and enriched in the perinuclear region, the triad, and abnormal intracellular aggregates. Splice-blocking ccdc78 morpholino results in changes mirroring the human disease, including abnormal RNA processing, altered motor function, and abnormal muscle ultrastructure. Morphants exhibit areas of swollen SR consistent with ryanodine receptor (RYR) related myopathies. Indeed, CCDC78 co-labels with RYR1, an essential component of the excitation-contraction coupling complex. To examine this process, we are using the calcium indicator GCaMP and have observed diminished calcium flux in morphants. Current data suggests this ccdc78 mutation disrupts the regulation of both excitation-contraction coupling and autophagy. To further determine the role of CCDC78 in normal muscle function, we have developed both morpholino based and stable genetic models (sa3254). In all, we have identified a novel gene that is the basis of congenital myopathy, developed a model to investigate the pathogenic mechanism, and are elucidating the function of CCDC78 in muscle physiology and development.

210. A Cell and Molecular Toolbox to Study Epithelia in Zebrafish. ***George T. Eisenhoffer***[1], *Hideo Otsuna*[2], *Justin Lopez*[3], *Daniel S. Wagner*[3], *Chi-Bin Chien*[2], *Richard I. Dorsky*[2], *Jody Rosenblatt*[1]. 1) Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT; 3) Department of Biochemistry and Cell Biology, Rice University, Houston, TX.
Epithelial tissues provide a protective barrier for our body and organs. The cells comprising epithelia are continually being eliminated by apoptosis and replaced by cell proliferation, yet the mechanisms that coordinately regulate cell loss and division to control overall numbers in these tissues are not well understood. Here we report a set of GAL4 enhancer trap lines that are expressed in subsets of discrete epithelial cell types in the developing zebrafish that can be used to investigate how cell proliferation and death are coordinated in a living tissue. The optical clarity of developing zebrafish and superficial nature of the epidermis make it ideal for imaging population dynamics and individual cell behaviors. When combined with UAS effector lines, our epithelial GAL4 collection allows for the visualization of specific cells for imaging, overexpression of genes of interest, and targeted ablation. We characterized the expression patterns of the different lines and determined their location with respect to the outer epithelial layer. These analyses revealed lines specific to a subset of the surface keratinocytes and basal progenitor cells, as well as specialized cell types. We induced death specifically within the different epithelial cell types and found essential roles for the basal and surface keratinocytes cells in epithelial development and maintenance. Our platform also provides the ability to use live imaging to monitor cell extrusion or proliferation in parallel with the induced cell death, allowing visualization of epithelial cell turnover and regeneration. Finally, we use photo-cleavable morpholino oligonucleotides directed against GAL4 to spatially and temporally control expression from the epithelial enhancer trap lines. Together, our system allows for cellular and molecular manipulation of a living vertebrate epidermis and will be of broad use to rapidly identify new mechanisms controlling epithelial maintenance, repair and carcinogenesis.

211. Drug screening for skeletal diseases using zebrafish models. *Roberta Gioia*[1], *Assunta Gagliardi*[2], *Velia Penza*[1], *Laura Bianchi*[2], *Francesca Tonelli*[1], *Silvia Carra*[3], *Franco Cotelli*[3], *Luca Bini*[2], *Marco Biggiogera*[4], *Sergey Leikin*[5], *Shannon Fisher*[6], *Antonio Rossi*[1], *Ruggero Tenni*[1], ***Antonella Forlino***[1]. 1) Department of Molecular Medicine, Biochemistry Unit, University of Pavia, Pavia, Italy; 2) Functional Proteomics Lab., Department of Life Sciences, University of Siena, Siena, Italy; 3) Department of Biosciences, University of Milano, Milan, Italy; 4) Department of Biology and Biotechnologies "L. Spallanzani", University of Pavia, Pavia, Italy; 5) Section of Physical Biochemistry, NICHD/NIH, Bethesda, Maryland, USA; 6) Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, USA.
The zebrafish skeletal system reproduces both membranous and endochondral bone formation and the key regulators of bone generation are highly conserved between teleosts and mammals. The classical form of the bone disease Osteogenesis Imperfecta (OI) is caused by dominant mutation in the COL1A1 and COL1A2 genes, coding for the collagen I alpha chains. Collagen type I in higher vertebrates it is a heterotrimer of two a1 and one a2 chains. In order to use zebrafish as OI model in drug screening it is necessary to know the exact composition of collagen type I that is unfortunately still poorly understood. In zebrafish the existence of three genes coding for three different a(I) chains, named a1, a2 and a3, has been described, but their stoichiometric association is unknown. To elucidate collagen I composition we purified acid and pepsin soluble collagen I from bone, skin and scales of adult fishes and from whole embryos at 2, 5 and 10 dpf. 1D and 2D electrophoretic analysis followed by mass spectrometry revealed in adult the comigration in a unique band/spot of a1

and a3 chains, while in embryos it was possible to distinguish an a1 and an a1/a3 comigrating band/spot. These data suggest the existence of [a1(I)]2a2(I) and a1(I)a2(I)a3(I) heterotrimers in embryos and of a1(I)a2(I)a3(I) in adults. The deep characterization of Chihuahua (Chi+/-), a zebrafish model for Classical OI carrying the G574D substitution in the a1(I) chain will allow us to use this fish for drug screening. Work supported by Fondazione Cariplo 2013-0612, Telethon GGP13098 and European Community n. 60230.

212. Genetic studies of stress response in zebrafish. ***Deepak Ailani***[1,2], *Pradeep Lal*[1,2], *Akira Muto*[1,2], *Koichi Kawakami*[1,2]. 1) Division of Molecular and Developmental Biology, National Institute of Genetics, Mishima, Japan; 2) Department of Genetics, Graduate University for Advanced Studies (SOKENDAI), Mishima, Japan.
The hypothalamic-pituitary-adrenal (HPA) axis is the major endocrine stress axis of vertebrates. Mammalian studies have shown that various brain structures such as limbic system structures and brainstem nuclei are involved in the regulation of HPA axis and glucocorticoid release. However, the neural mechanisms of such regulation are still largely unknown.

To address this question, we aim to identify functional neural circuits regulating HPA axis and stress response through genetic analysis using zebrafish. We used a swimming behavior of an isolated fish when introduced into a novel tank as a measure of anxiety. Anxious fish spend more time near the bottom.

Our enhancer trap and gene trap screens generated several transgenic zebrafish which express Gal4 in the specific regions of the adult zebrafish brain. Through systematic inactivation of Gal4 expressing neurons using UAS: neurotoxin fish, we analyzed the behavior in a novel tank, and identified a transgenic line that showed reduced stress response. In this line, Gal4 was expressed in the putative eminentia thalamus (EmT) in the adult brain. Anatomical studies have proposed EmT as functional homologue of mammalian Bed Nucleus of the Stria Terminalis (BNST), which is an important regulator of HPA axis in mammals. Thus, our study revealed a key neural population in adult zebrafish brain that regulates stress response. To understand the function of EmT in stress response, we are currently aiming to conduct anatomical and molecular studies. We are also establishing a system to study stress response in zebrafish larva that enables functional imaging using optogenetic tools.

213. Identification of Coordinate Regulatory mRNA/miRNA Modules for Connectivity Development. *JH Son*[1], *B Milash*[2], *TJ Stevenson*[1], *TM Dahl*[1], ***JL Bonkowsky***[1]. 1) Dept Pediatrics, U. Utah, Salt Lake City, UT; 2) Bioinformatics Shared Resource, HCI, U. Utah, Salt Lake City, UT.
While individual molecular pathways governing axon guidance and synapse formation are well described, little is know about how this connectivity development is coordinately regulated. We hypothesized that evolutionarily conserved “modules” of axon guidance and synapse formation genes, together with their interacting miRNAs (micro-RNAs), control connectivity development. We performed RNAseq analysis at different developmental stages. To identify coordinate regulation, we compared expression profiles in normoxia compared to hypoxia, reasoning that genes with similar developmental roles would exhibit similar changes in expression. We analyzed genes (mRNAs) involved in axon pathfinding and synapse development and function; and miRNA changes. We curated mRNAs based on known or putative roles in connectivity development using GO term analysis. Zebrafish genome annotation Zv9 was used for identifying mRNA reads. miRNA reads were identified using miRDeep software and sequences searched against miRBase version 17. Comparisons were made of expression of mRNAs; of miRNAs; and of mRNAs to miRNAs relationships. We used DESeq software (Bioconductor.org) to provide variance-corrected read counts. For each miRNA/mRNA pair we calculated a Spearman rank correlation between the expression profiles of a given miRNA and mRNA (or mRNAs). Strong anti-correlation (decreased expression of a mRNA corresponding to increased expression of a miRNA) between the expression profiles indicated genes likely to be regulated by a given miRNA. We found that subsets of mRNAs were coordinately regulated in different developmental epochs, with expression profiles that were inversely related to expression of distinct miRNAs. Based on their expression profiles, the connectivity genes fit into 10 distinct clusters. GO analysis demonstrated that the clusters were composed of specific gene types (for example, ion channels). Our results indicate that connectivity development is coordinately regulated across development, and reveals modules of gene expression, that are controlled in part through miRNAs.

214. Defective Migration of Facial Branchiomotor Neurons Reduces Food Intake in Zebrafish Larvae. *J. Allen*[1,3], *K. Bhattacharyya*[1,4], *J. Davis*[1], *K. Shafer*[1], *J. Cox*[2], *M. Voigt*[2], *J. Viator*[1,5], ***A. Chandrasekhar***[1]. 1) Univ of Missouri, Columbia, MO; 2) Saint Louis Univ Sch of Med, St. Louis, MO; 3) Washington Univ Sch of Med, St. Louis, MO; 4) Northwestern Univ, Evanston, IL; 5) Duquesne Univ, Pittsburgh, PA.
Feeding involves the coordinated action of eyes and jaws, and associated neural networks. The activity of the neural networks controlling jaw movements (branchiomotor circuits) is regulated by the visual, olfactory and hypothalamic systems, which are physiologically well characterized. In contrast, the physiological functions of the branchiomotor circuits, and the consequences of disruption of these circuits are poorly understood. To address these issues, we sought to measure the feeding ability of zebrafish larvae, a direct output of the branchiomotor circuits. We developed a robust qualitative method for evaluating food intake that allowed us to compare the feeding ability of control and experimental larvae at 7 days post fertilization. To validate our approach, we examined the effects of ablating large subsets of branchiomotor neurons using optical and chemical methods. As expected, elimination of a majority of trigeminal and facial branchiomotor neurons by metronidazole treatment of *Tg(zCREST:nitroreductase-mCherry)* larvae resulted in a significant decrease in food intake. Furthermore, laser-mediated ablation of trigeminal motor neurons also resulted in a significant reduction in food intake, indicating that the food intake assay is robust and sensitive. To study the consequences of defective migration, we examined food intake in *off limits* mutant (*fzd3a-/-*) larvae where facial branchiomotor neurons fail to migrate out of rhombomere 4 to their normal locations in rhombomeres 6 and 7. Mutant larvae exhibited a significant decrease in food intake. Importantly, other factors like swimming that might

influence feeding were not affected. These results suggest strongly that dislocation of facial branchiomotor neurons can lead to functional deficits in the circuits they serve. Our studies establish a foundation for dissecting the neural circuits driving a motor behavior essential for survival.

215. Generation and characterization of a zebrafish model of SLOS. ***Celine V. M. Cluzeau***[1*], *Alexa Ely*[1], *Abigail Cahn-Gambino*[1], *Oakleigh M. Folkes*[1], *Christopher A. Wassif*[1], *Benjamin Feldman*[2], *Forbes D. Porter*[1]. 1) PDEGEN, NICHD, NIH, DHHS, Bethesda, MD; 2) PGD, NICHD, NIH, DHHS, Bethesda, MD.
Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder characterized by multiple malformations, cognitive impairment and abnormal behavior including autistic traits. It is caused by mutations of the *DHCR7* gene, encoding the enzyme 7-dehydrocholesterol (7DHC) reductase. No therapy is available to date. Several rodent models were useful for elucidating some aspects of this syndrome, but they do not offer the ability to study postnatal brain development. We hypothesized that the cholesterol-rich zebrafish yolk would allow the survival of *dhcr7*-deficient embryos, and provide an alternative model to study the mechanisms responsible for SLOS neurological phenotype. We produced a novel SLOS zebrafish model using TALENs. Six frameshift and three in-frame mutations in two different exons of *dhcr7* were selected to derive lines. We showed that all mutant lines present the characteristic accumulation of 7DHC and decreased cholesterol content in both liver and brain from 3 weeks of age, with variable severity depending on the mutation. *dhcr7*-deficient fish growth is delayed. Mutant fish are able to breed. However, the yolk from eggs produced by mutant females contains 7DHC, and the 1-week-old mutant progeny of the mutant females already present with high levels of 7DHC. Despite this early exposure to 7DHC, these mutants survive past 7 weeks of age. However, preliminary results of cartilage staining in one-week-old larvae showed that about one third of these mutants have jaw anomalies. Initial behavioral studies of *dhcr7*-deficient larvae suggest that mutants' movement is decreased relative to control and heterozygous larvae. We are working on confirming these results, as well as determining whether this decreased activity reflects a general developmental delay or a specific behavioral defect. In either case, it might be a useful trait to develop an in vivo assay for screening potential therapeutic drugs. Future work will continue the characterization of the development, motor and cognitive functions as well as the behavior of *dhcr7*-deficient fish at larval, juvenile and adult stages.

216. Short wavelength violet light stimulates melanopsin-dependent alertness responses in zebrafish. ***Jorge E Contreras***[1], *Thomas Lisse*[2], *Jessie Rottersman*[2], *Chifaa Bouzidi*[2], *Ann Cavanaugh*[3], *Elizabeth Brochu*[2], *Sandra Rieger*[2]. 1) Department of Pharmacology & Physiology, Rutgers New Jersey Medical School, Newark, NJ; 2) Department of Regenerative Biology and Medicine, Mount Desert Island Biological Laboratory, Salisbury Cove, ME; 3) Department of Molecular, Cell and Developmental Biology, University of California Los Angeles, Los Angeles, CA.
Sunlight throughout the day and seasons strongly influences our biological rhythms and activity. In recent years it has become evident that nighttime overexposure to bright light in urban environments can profoundly affect physiology and behaviour in humans and animals. In particular, artificial emission of high contents of short-wavelength light has been shown to stimulate alertness in humans but the mechanisms remain largely unknown. Utilizing a larval zebrafish model, we identified acute, non-image forming responses to the short-wavelength violet light that are reminiscent of alertness, including increased heart rate, locomotor activity and pectoral fin beating (for increased oxygen supply). We further determined that these responses are driven by sympathetic neuronal circuits and depend on the zebrafish melanopsin homolog, Opn4a. These responses can be modulated by the sleep-regulatory hormone melatonin, but this is not essential for triggering the violet light response. Our findings reveal a novel mechanism of violet light-induced alertness.

217. Behavioral recovery from an aversive stimulus corresponds with left habenular activity. ***Erik R. Duboué***[1], *Akira Muto*[2], *Koichi Kawakami*[2], *Marnie Halpern*[1]. 1) Carnegie Institution for Science, Baltimore, MD; 2) Molecular and Developmental Biology, National Institute of Genetics, Mishima, Shizuoka, Japan.
Throughout vertebrates the bilaterally paired habenular nuclei link the limbic forebrain with midbrain targets. Disruption of the dorsal habenular nuclei (analogous to the mammalian medial habenulae) has been correlated with increased fear/anxiety-related behaviors. In larval zebrafish, the dorsal habenulae exhibit prominent left-right differences in size, sub-nuclear and neuropil organization, and in connectivity with their shared target, the interpeduncular nucleus (IPN). We sought to determine how habenular asymmetry might influence behavioral responses by exposing 7-8 dpf larval zebrafish to an aversive stimulus, electrical shock (5V/cm^2; 5 pulses; 1 pulse per sec). In freely moving wild-type (*situs solitus*) larvae, a mild electric shock results in a short period (~10 sec) of reduced activity followed by a return to baseline. In contrast, larvae with reversed habenular asymmetry (*situs inversus*) show a significantly longer period of immobility (~40 seconds) or enhanced freezing. To explore the respective contributions of each habenula in response to shock, we also assayed larvae with left or right isomerization of the habenular region. Larvae with left isomerization showed no significant difference from their WT siblings. However, larvae with right isomerization exhibited an extended period of freezing following shock similar to those with L-R reversed habenulae. To investigate whether neural activity in the habenular nuclei influences the response to shock, the heads of larvae expressing a genetically encoded calcium indicator were immobilized in agar. Electrical shock (5V/cm^2; 1 pulse per sec; 1 pulse) was applied under a confocal microscope so that neural activity and locomotor responses could be simultaneously monitored in the head affixed larvae. We found that behavioral recovery from shock temporally correlates with an increase in neural activity in a subregion of the left habenula. The results demonstrate that fear/anxiety-related behavior can be assayed in larval zebrafish and reveal a preferential role for the left habenula in recovery from an aversive stimulus.

218. Effective Q transcriptional regulatory system for modulation of gene activity and gene trapping in zebrafish. ***Antara Ghosh****, Estela Monge, Michelle Macurak, Abhignya Subedi, Marnie E Halpern.* Department of Embryology, Carnegie Institution for Science, Baltimore, MD.
The functionality of the Q regulatory system of Neurospora crassa has been demonstrated in Drosophila, C. elegans, mammalian cells and zebrafish. Previously, we reported the generation of tissue-specific QF driver lines and a QUAS:GFP reporter (Tg[QUASR:GFP]c403 where R signifies reversed orientation of the QUAS) that has been maintained for six generations without evidence of diminished labeling due to silencing. In addition, we established a new QUAS:GFP line from a vector that has the 5xQUAS placed in correct orientation to the gfp gene. Embryos derived from females bearing the Tg[QUAS:GFP;bcrys:CFP]c469 insertion do not show maternal deposition of GFP, as is observed with the c403 line. As in Drosophila, QF is toxic when expressed in high doses in zebrafish. We also assayed a codon optimized and truncated QF, QF2; (C. Potter, pers. comm.) that retains activity but shows significantly reduced toxicity in transgenic flies. QF2 is not only less toxic but is also a highly effective activator of transcription in zebrafish. For increased versatility, we adapted the split QF system used in C. elegans, in which the QF activation and DNA binding domains are separated, modified with leucine zipper sequences, and expressed from different transgenes. Following co-injection of the two Tol2 vectors into 1-cell stage embryos, we found that the two peptides could reassemble to activate a QUAS:GFP transgene. We are currently testing this method for intersectional approaches in stable transgenic lines. For maximal utility, it is important to have a collection of QF tissue-specific transgenic drivers. To accomplish this efficiently, we produced gene trap vectors using the original QF transcription factor as well as QF2. In initial screening, founders have been identified with insertions that activate QUAS:GFP in discrete patterns in the developing brain. In summary, the Q system provides a reliable and powerful alternative for control of gene activity in zebrafish. All components have been introduced into Gateway vectors modified for Tol2 transposition for ease of use by other laboratories.

219. Single cell resolution neuroarchitectural map of hypothalamic stress circuits. ***Ulrich Herget****, Jose Arturo Gutierrez-Triana, Luis Castillo-Ramirez, Soojin Ryu.* Max Planck Institute for Medical Research, Heidelberg, Germany.
A neuron's functional identity is determined by both its morphological and biochemical features. Yet, it has been difficult to define for a given neural circuit the comprehensive neuronal architecture that includes both of these features at single cell resolution. In this study, we focused on the hypothalamic neural circuits that mediate the stress response, and established a neuroarchitectural map based on the neuropeptide expression and morphology of individual cells. The stress response is a set of behavioral and physiological responses to any threat to homeostasis, and is controlled by neurons in the hypothalamic paraventricular nucleus (PVN) in mammals. Due to its position deep within the mammalian brain, key anatomical and functional features of many PVN neurons are poorly understood. Here we 1) defined the exact location and borders of the neurosecretory preoptic area (NPO) as the PVN-homologous region in the larval zebrafish brain and 2) characterized neurochemical and morphological properties of NPO neurons at single cell resolution. To determine neurochemical identities of individual NPO cells, we first generated a comprehensive 3D map of expression patterns of 9 neuropeptides (arginine vasopressin, corticotropin-releasing hormone, proenkephalin a/b, neurotensin, oxytocin, vasoactive intestinal peptide, cholecystokinin, and somatostatin). Next, we determined the degrees of co-expression of two neuropeptides in the same cell by performing systematic pairwise comparisons. In order to combine this neurochemical identity with morphological information, we employed the Brainbow technique. To this end, we generated a transgenic Brainbow line using a regulatory element from the orthopedia gene labeling NPO cells. We then combined 3D reconstructions of single cell morphology with neuropeptide identity analysis. Our results allowed the classification of cells in the NPO into distinct morphological types. Within each morphological type, heterogeneity of cell types based on neuropeptide expression was apparent. Thus, this work provides a critical basis for the analysis of the development, function, and plasticity of neuronal stress circuits in larval zebrafish.

220. Ion channels TRPM1 and TRPM7 in melanocyte development. ***Greg A. Bonde***[1]*, Amanda Decker*[1]*, Pavani Beesetty*[2]*, Ashot Kozak*[2]*, Robert Cornell*[1]. 1) The University of Iowa, Iowa City, IA; 2) Wright State University, Dayton, OH.
The Transient Receptor Potential Melastatin-like 7 (TRPM7) is unusual among ion channels because a) it is permeable to magnesium, b) it contains an intrinsic kinase domain, and c) it is necessary for cellular differentiation. Here we seek to determine how the first two features relate to the last. In zebrafish *touchdown/touchone/nutria* mutants, all corresponding to loss-of-function alleles of *trpm7,* embryonic melanocytes exhibit abnormal differentiation and undergo non-apoptotic cell death. By contrast, regeneration melanocytes, which are stem-cell derived, are not obviously compromised in *trpm7* mutants, implying the existence of a compensatory protein in them. There are 8 members of the TRPM subfamily. A candidate to provide compensatory function in regeneration melanocytes is Trpm1a, which is expressed in embryonic and regeneration melanocytes; however, Trpm1a lacks a kinase domain. To identify the functional components of Trpm7 that are necessary for melanocyte development we are testing the ability of engineered variants of Trpm7, and wild-type Trpm1a, to rescue melanocytes in *trpm7* mutants. To learn the role of Trpm1a in regeneration melanocytes we have generated a crispr/cas mediated knock-out of *trpm1a* and are currently raising F0 animals. Fruit flies possess a single Trpm ortholog. Voltage-clamp experiments in tissue culture cells transfected with cDNAs encoding zebrafish Trpm7 or fruit-fly Trpm indicate the two channels have very similar current/voltage relationships. This implies the biochemical function of Trpm7 in melanocyte differentiation may be an ancient rather than an evolutionarily novel one. To test this possibility we are testing whether Drosophila Trpm can substitute for Trpm7 in melanocyte differentiation. The results of these experiments will yield a better understanding of mechanism and the evolutionary history of a novel pathway essential for melanocyte differentiation and survival. Such pathways are relevant to inherited disorders of pigmentation and metastatic melanoma.

221. Investigation of ethanol induced axon pathfinding defects in the central nervous system. *Aaron Beck, Andrew Ross, Cecilia Culp, Aleksander Krazinski, Jiakun Fu, Leah Shorser-Gentile, Roland Watt, Camille Yongue,* ***Jennifer Bonner***. Biology, Skidmore, Saratoga Springs, NY.
In humans, in utero exposure to ethanol can lead to Fetal Alcohol Spectrum Disorder (FASD). Individuals affected by FASD experience a variety of symptoms that include impaired motor function, physical deformities, and cognitive deficits. As a disorder that comprises disparate outcomes, the mechanisms that underlie nervous system dysfunction in FASD are incompletely understood. In this study, low doses of ethanol were used to elucidate specific interactions between ethanol and axon pathfinding, in an attempt to avoid secondary effects of morphological disruptions observed when higher doses of ethanol are utilized. Embryonic zebrafish were exposed to 50-80 mM or (0.3%-0.48 %) ethanol at 2-4 hpf, until the termination of the experiment, between 24 and 36 hpf. To determine what, if any, CNS neurons are misrouted as a result of ethanol exposure, spinal neuron pathfinding was analyzed using znp-1, anti-acetylated tubulin, and 3A10 immunofluorescence. Conventional and confocal fluorescent microscopy was employed. Embryos exposed to ethanol continuously during development exhibit shortened motoneuron axons as well as excess branching. When embryos are exposed to ethanol later in development (during axon outgrowth), motoneurons develop normally, suggesting that early developmental exposure to ethanol is critical for observed defects.

222. Zebrafish Tauopathy Models for Translational Research. *Qing Bai[1], Yanzhong Zhou[1,3],* ***Edward A Burton***[1,2]. 1) University of Pittsburgh, Pittsburgh, PA; 2) Geriatric Research, Education and Clinical Center, Pittsburgh VA Healthcare System, Pittsburgh, PA; 3) Tsinghua University, Beijing, China.
Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are common neurodegenerative diseases associated with prominent motor and cognitive abnormalities and a poor prognosis (median survival 6 - 8 years). Both diseases are characterized by neuronal loss and accumulation of abnormal aggregates of the 4-repeat isoform of the microtubule-associated protein Tau (4R-Tau) in neurons throughout the CNS. It is thought that 4R-Tau is central to the pathogenesis of these disorders, because mutations (e.g. P301L) in the MAPT gene encoding Tau can give rise to PSP and CBD phenocopies, and PSP and CBD are strongly associated with genetic variants at the MAPT locus. In order to address a critical barrier to progress in developing drugs to target Tau accumulation and its consequences in neurons in vivo, we have developed zebrafish Tauopathy models. Our earlier work showed that transgenic zebrafish expressing a human 4R-Tau transgene constitutively in CNS neurons developed progressive neurological phenotypes during aging, associated with Tau hyperphosphorylation, similar to the human diseases. In order to develop models that are optimized for drug discovery, rapid comparative testing of putative therapeutic agents, and studies to identify novel therapeutic targets, we have now generated transgenic lines expressing human Tau conditionally at high levels using the bipartite Gal4/UAS system. In distinction to previously reported zebrafish Tau models, the enhanced models we have developed show impaired survival, motor abnormalities demonstrable using automated video tracking in multiwell plates, oculomotor abnormalities (a hallmark of PSP), and histopathological changes representative of human disease including somatodendritic accumulation of hyperphosphorylated Tau. Since these abnormalities occur over a short time course , these novel transgenic lines will be a valuable tool for translational studies to develop new therapies for PSP and CBD, and potentially other Tauopathies such as Alzheimer's disease and chronic head injury.

223. The zebrafish as a model system to study human CAMTA1-related hereditary ataxia. ***C. Cianciolo Cosentino***[1], *D. Loffing-Cueni*[2], *S. Neuhauss*[1], *J. Loffing*[2]. 1) Institute of molecular life sciences, University of Zurich, Zurich, CH; 2) Institute of Anatomy, University of Zurich, Zurich CH.
Hereditary ataxias (HA) are a wide and heterogeneous group of neurological disorders with cerebellar neuron degeneration as a common hallmark. HA are phenotypically characterized by motor incoordination, developmental delay, instability of posture, hypotonia, and/or visual problems. The genetic cause and the underlying pathophysiological mechanism for many forms of HA are still unclear. Recent studies (Theveron et al., 2012) have shown that loss-of-function mutations in the calmodulin-binding transcription activator 1 (CAMTA1), a calcium responsive transcription factor, are associated with HA and mental retardation in humans. In an attempt to establish a zebrafish model of HA, we cloned the zebrafish CAMTA1 ortholog camta1a. The deduced amino acid sequence display a 64% overall amino acid identity with human CAMTA1. Expression analyses in zebrafish larvae showed brain-specific camta1a expression, restricted to the optic tectum, the cerebellum and the hindbrain. The injection of translation blocking camta1a antisense morpholino oligonucleotides reproduced the main symptoms of ataxia. In particular, camta1a morphants presented abnormal body movement, with increased spontaneous contractions, shorter swimming path, and disability to maintain upright posture. Moreover, camta1a MO larvae showed a reduction in Purkinje cells markers, indicating a possible degeneration of Purkinje cells. Interestingly, mice with targeted inactivation of Camta1 gene in parvalbumin positive cells also recapitulate salient features of ataxia, and further histological analyses showed a significant reduction of Purkinje cells in the cerebellum of Camta1 conditional mutant mice. Taken together, our results validate zebrafish as a valuable model organism in which to investigate the pathophysiological mechanisms underlying the disease phenotype in humans with CAMTA1 mutations.

224. Schwann Cell Morphology and Function During Peripheral Nerve Regeneration. ***Melissa M. Ducommun****, Michael Granato.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
In the peripheral nervous system, axons damaged by injury have the ability to regrow and reconnect to their original targets. Schwann cells are the main glia of the peripheral nervous system and are critical for axonal maintenance and regeneration. Following injury, Schwann cells dedifferentiate to an immature state that promotes axonal regrowth and nerve repair. The process of Schwann cell dedifferentiation is accompanied by drastic morphological changes as well as cell migration. However, exactly how these morphological changes are driven by intracellular events, such as cytoskeletal rearrangements and membrane remodeling via intracellular transport, remains unknown. We have recently established an in vivo assay to laser-transect GFP-expressing motor nerves in live intact zebrafish larvae, and to subsequently

observe at a minute-by-minute resolution the processes of axonal de- and regeneration (Rosenberg et al 2012). Using this assay, I am determining how cytoskeletal and intracellular transport dynamics in Schwann cells change in response to nerve injury. As a first step to probe the requirement for intracellular transport in axonal regeneration, I assayed regeneration in mutants for the motor protein dynein. I found that both homozygous but also heterozygous dynein mutants exhibit impaired axonal regrowth, indicating a requirement for dynein during axonal regrowth in vivo. Importantly, axonal development and Schwann cell development or number are unaffected in heterozygous dynein mutants, providing the unique opportunity to study the role of dynein in regeneration without complication due to developmental deficits. Recent studies have shown that dynein is required in Schwann cells for proper differentiation and myelination during development. This suggests that besides its well-established role in neurons, dynein may also play an important role in Schwann cells after injury and during axonal regeneration. I am using a cell-type specific rescue approach to test whether dynein is required in Schwann cells during axonal regeneration. I will present results of these ongoing experiments to determine the role of cytoskeletal rearrangements and intracellular transport in Schwann cells after nerve injury.

225. Detailed analysis of neural regeneration through Dynamic imaging *in vivo*. ***Timo Friedrich***, *Yona Goldshmit, Jean Tang, Frisca Frisca, Daniel Colquhoun, Graham Lieschke, Jan Kaslin.* Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia.
In contrast to most mammalian model organisms, larval zebrafish are capable of regenerating massive damage to their brain or spinal cord within days after injury. Yet, the underlying mechanisms that regulate neurogenesis and regeneration of the vertebrate brain remain unclear. We have developed an imaging assay to dynamically study spinal cord regeneration at single cell resolution *in vivo*. Using this imaging assay and genetic lineage tracing after spinal cord injury we have discovered that the plasticity of the glial cells is one of the major cellular mechanisms contributing to neural recovery in zebrafish. Glial cells exhibit a dual role in neural regeneration by serving as stem cells to replenish lost cells and as scaffolds to form pioneering processes, “bridges”, which span the lesion and initiate the regeneration process. The driving molecular factors that initiate directional outgrowth and bridging of processes and subsequent cell migration is not known. One plausible hypothesis is that immune cells and inflammatory cytokines mediate this response. We recently showed that inflammation is required and sufficient to kick start and boost the regenerative response in the zebrafish brain. In accordance with this, a massive infiltration of neutrophils and macrophages is detected during the first hours after spinal cord injury. To test this hypothesis, we ablated neutrophils and macrophages using the genetically encoded nitro reductase system and found that to be required for spinal cord recovery. To test specific pathways involved in the recovery process after spinal cord injury, we used a zebrafish mutant with a defect in *sprouty*, an FGF inhibitor. Increased FGF signaling improved the time required for glial bridge formation. Taken together, the dynamic analysis of chronology, the immune cell response and ability to quickly screen for beneficial effects on spinal cord recovery offer a unique window in to the understanding of spinal cord injury recovery mechanisms. This approach can be used for future genetic and pharmacological screening for novel strategies to improve recovery.

226. Genetic Influences on Zebrafish Enteric Nervous System Development. ***Julia Ganz***, *Ellie Melancon, Angel Amores, Peter Batzel, Marie Strader, Ingo Braasch, John Postlethwait, Judith Eisen.* Institute of Neuroscience, 1254 University of Oregon, Eugene, OR, USA.
The enteric nervous system (ENS) regulates essential gut functions including motility, secretion, and homeostasis and is composed of diverse neuronal subtypes and glia. Abnormal ENS development causes human diseases, for example Hirschsprung disease (HSCR), in which the distal gut is uninnervated and nonmotile. HSCR is a multifactorial disease and although causative mutations are known in some cases, there are many cases in which the genetic basis remains unknown. We identified zebrafish mutant *b1074* from a genetic screen based on a reduction in ENS neurons. To further characterize the mutant phenotype, we quantified enteric neurons in the gut mid-intestine and vent. Similar to human HSCR patients, *b1074* mutants have a variable reduction of ENS neurons near the vent and a mild reduction in the mid-intestine. Reduction of ENS neurons could result from a decrease in ENS progenitors and/or abnormal progenitor migration. We compared the number of Foxd3 positive ENS progenitors in *b1074* mutants and wild-type siblings and found that mutants have significantly fewer ENS progenitors in the vent and mid-intestine. We also analyzed ENS progenitor migration by in vivo imaging and analysis of enteric progenitor position along the gut at different developmental time points and found there is a significant delay of *phox2b* positive ENS progenitor migration in *b1074* mutants compared to wild-type siblings. Thus, *b1074* shows a variable reduction in the number of ENS neurons which may be caused by a decreased progenitor pool and abnormal progenitor migration. Using RAD-tags and whole-genome sequencing, we identified a 1 Mb interval on chromosome 5 with a non-synonymous SNP in our candidate gene, which has not been previously implicated in ENS development. With its variable phenotype and novel candidate gene, *b1074* serves as an exciting new HSCR model.

227. Myosin phosphatase activity controls axon guidance and motoneuron cell body positioning. ***Juliane Bremer***, *Michael Granato.* University of Pennsylvania, Department of Cell and Developmental Biology, Philadelphia, PA.
A critical intracellular molecular motor during cell migration and directed axon outgrowth is non-muscle myosin II (myosin II). Myosin II is targeted by the repulsive axon guidance molecules slit, netrin-1 and semaphorin 3A to mediate growth cone collapse and axon retraction. By phosphorylation of the myosin light chain, myosin light chain kinase (MLCK) and Rho-kinase can both activate myosin II, and both kinases have been implicated in repulsive axon guidance. Dephosphorylation of myosin II decreases myosin II function, and is regulated by the myosin phosphatase complex. Surprisingly, the role of the myosin phosphatase complex and the consequences of enhanced myosin II activation on axon guidance are not well understood. We previously isolated a mutant with axon guidance errors from a forward genetic screen, and have now identified a causative non-sense mutation in the myosin binding subunit of myosin phosphatase, *ppp1r12a*. This C1316A mutation causes a premature stop after 438 of 1049 amino acids and is predicted to reduce or abolish myosin phosphatase function, predicted to enhance myosin II activity. We find that in *ppp1r12a* mutants, motor axons pathfind properly until the first axons reach the horizontal myoseptum and then exhibit excessive branching and/or stall in 58% of the hemisegments. Moreover, we find that the

cell body position of 33% of CaP motoneurons is shifted rostrally. Using chimera analysis, we demonstrate that *ppp1r12a* function in motoneurons is dispensable for axonal morphology, while *ppp1r12a* acts cell autonomously in CaP motoneurons for proper soma positioning. Using live cell imaging, we find that in *ppp1r12a* mutants, motoneurons exhibit membrane blebbing, a sign of increased myosin II activity. This blebbing occurs prior to axon outgrowth, yet motoneurons position properly at this stage and only shift cell body position after axonogenesis is well under way. Thus, our studies demonstrate that *ppp1r12a* plays multiple independent roles in motoneuron development and suggest a novel role for myosin phosphatase activity in neuronal cell body positioning. We will further present results of ongoing studies to dissect myosin II localization and phosphorylation during motoneuron development.

228. Investigating an attractive role for Repulsive Guidance Molecule A (RGMa) in neural tube morphogenesis. ***Sharlene P. Brown***, *Valerie N. Olmo, Pradeepa Jayachandran, Rachel M. Brewster.* Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD.
Repulsive guidance molecule A (RGMa) is a member of the RGM family of guidance molecules with a growing repertoire of functions in the developing and adult nervous system. These cell surface proteins are GPI-anchored and can therefore be cleaved off of the membrane for long range signaling. As its name indicates, RGMa was initially identified based on its repulsive effect on growing neuronal cells. Recent studies from our laboratory have revealed that depletion of RGMa and/or its putative ligand, Neogenin (Neo), prevents polarized migration and cell elongation during convergent extension (CE) of the neural ectoderm, resulting in a widened neural plate. This observation indicates that, in this context, RGMa functions to promote rather than inhibit cellular extensions. Intriguingly, RGMa is expressed at high levels in the dorsal midline (notochord), where it may potentially function as a signal to attract migrating cells. To test this hypothesis we have misexpressed RGM away from the midline, in the neural ectoderm. Preliminary data indicates that neural cells in the vicinity of ectopic RGMa preferentially extend membrane protrusions towards this new source. These data point to a context-dependent activity of RGMs in neural development. Furthermore, the severe neural tube defects observed in mouse and *Xenopus* RGMa loss of function embryos suggest a conserved role for this pathway in mediating neural tube morphogenesis.

229. *SCCPDH* regulates early brain development in zebrafish. ***Elizabeth A. Burke***[1,2], *William A. Gahl*[2], *Cornelius F. Boerkoel*[1]. 1) NIH Undiagnosed Diseases Program Translational Laboratory, Bethesda, MD; 2) Section on Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.
SCCPDH is a human nuclear envelope transmembrane-associated protein that currently has no known function. *SCCPDH* lies within the critical region for the 1q44 microdeletion syndrome, which is characterized by intellectual disability, developmental delay and brain anomalies. We hypothesized therefore that SCCPDH contributes to brain development. Testing this by morpholino knockdown of the zebrafish homologue, we observed a dose-dependent loss of brain tissue at the midbrain-hindbrain boundary, decreased eye size, and hydrocephalus. This morphant phenotype was rescued by co-injection of wild type *SCCPDH* mRNA in over 80% of embryos. Overexpression of wild type mRNA in zebrafish resulted in an increased incidence of cyclopia and abnormal forebrain development. *In situ* hybridization and gene expression array analyses of *SCCPDH* morphants found deregulated gene expression including overexpression of *pax2* in the optic primordia and reduced expression of *dlx2a*, *otx2*, and *gsc* in the forebrain and midbrain at 24 hours post fertilization. These results confirm a role for SCCPDH in brain formation and suggest that SCCPDH modulates the expression of key developmental pathways.

230. Investigation of spinal cerebrospinal fluid-contacting neurons expressing PKD2L1: evidence for a conserved system from fish to primates. ***Lydia Djenoune***[1,2], *Hanen Khabou*[1], *Fanny Joubert*[3], *Feng B. Quan*[2], *Sophie Nunes Figueiredo*[1], *Laurence Bodineau*[3], *Filippo Del Bene*[4], *Céline Burcklé*[1,5], *Hervé Tostivint*[2], *Claire Wyart*[1]. 1) Institut du Cerveau et de la Moelle Épinière (ICM), INSERM UMR 1127, CNRS UMR 7225, UPMC Univ Paris 06, Paris, France; 2) Muséum National d'Histoire Naturelle, CNRS UMR 7221, Paris, France; 3) UPMC Univ Paris 06, F-75005, INSERM UMR S 1158, Paris, France; 4) Institut Curie, CNRS UMR 3215, INSERM U 934, Paris, France; 5) Institut de Biologie du Développement de Marseille, Marseille, France.
Over ninety years ago, Kolmer and Agduhr identified spinal cerebrospinal fluid-contacting neurons (CSF-cNs) based on their morphology and location within the spinal cord. In more than two hundred vertebrate species, they observed ciliated neurons around the central canal (cc) that extended a brush of microvilli into the cerebrospinal fluid. Although their morphology is suggestive of a primitive sensory cell, their function within the vertebrate spinal cord remains unknown. The identification of specific molecular markers for these neurons would benefit the investigation of their physiological roles. PKD2L1, a transient receptor potential channel that could play a role as a sensory receptor has been found in cells contacting the cc in mouse. Here we demonstrate that PKD2L1 is a specific marker for CSF-cNs in the spinal cord of mouse, macaque and zebrafish. In these species, the somata of spinal PKD2L1+ CSF-cNs were located under or within the ependymal layer and extended an apical bulbous extension into the cc. We found GABAergic CSF-cNs expressing PKD2L1 in all three species. We took advantage of the zebrafish embryo for its transparency and rapid development to identify the progenitor domains from which pkd211+ CSF-cNs originate. pkd2l1+ CSF-cNs were all GABAergic and organized in two rows—one ventral and one dorsal to the cc. Their location and marker expression is consistent with Kolmer-Agduhr cells (KAs). Accordingly, pkd2l1+ CSF-cNs were derived from the progenitor domains p3 and pMN defined by the expression of nkx2.2a and olig2 transcription factors respectively. Altogether our results suggest that a system of CSF-cNs expressing PKD2L1 is conserved in the spinal cord across bony vertebrate species.

231. Pericentriolar Material 1 (PCM1) regulates the asymmetry of Mind bomb and Notch signaling during asymmetric division of neural stem cells. ***Z. Dong***[1], *A. Kodani*[2], *D. Yu*[3], *X. Shu*[3], *J. Reiter*[2], *S. Guo*[1]. 1) Pharmaceutical and Bioengineering Sciences, UCSF, San Francisco, CA; 2) Biochemistry & Biophysics, UCSF, San Francisco, CA; 3) Pharmaceutial Chemistry, UCSF, San Francisco, CA.
Asymmetric division is a robust means to control self-renewal and differentiation of radial glia (RG) progenitors, the principal neural stem cells (NSCs) in the vertebrate brain. How asymmetric division is regulated in vivo in vertebrate systems remains incompletely understood.

We have previously shown that during active neurogenesis, an individual NSC can divide asymmetrically to generate basal self-renewing and apical differentiating daughters. The cell fate determinant Mind bomb (Mib) unequally segregates to the apical daughter, thereby restricting high Notch activity and self-renewal potential to the basal daughter. Here, through immunohistochemistry and live imaging in the intact zebrafish brain, we find that both Mib and the Notch ligand Dld show dynamic centrosomal localization during the cell cycle. Biochemical analysis reveals that Mib interacts with the centrosomal protein Pericentriolar Material 1 (PCM1). Strikingly, the asymmetry of Mib and Notch signaling is disrupted in the paired daughter cells of pcm1 morphants. These findings suggest that PCM1 is a key regulator of asymmetric Notch signaling in NSC self-renewal and differentiation.

232. Anillin links cytokinesis to mode of cell division in the retinal neuroepithelium. *Alessio Paolini[1], **Anne-Laure Duchemin**[1], Shahad Albadri[1], Vincenzo Di Donato[2], Filipo Del Bene[2], Flavio Zolessi[3], Lucia Poggi[1]*. 1) Centre for Organismal Studies, Heidelberg, Baden-Württemberg, Germany; 2) Pôle de Biologie du Développement et Cancer, Institut Curie, France; 3) Sección Biología Celular, Facultad de Ciencias, Universidad de la República, Uruguay.
Generation of neuronal diversity in the vertebrate neuroepithelium requires coordinated regulation of cell polarity, cell division, and cell fate determination. The complex networks integrating these processes in vivo remain elusive. Here we use the retinal ganglion cell (RGC) progenitors as model for asymmetric cell division in the developing retinal neuroepithelium of the zebrafish embryo. We find that the apical domain of one daughter cell unequally inherits apical membrane components such as Par3 and F-actin during asymmetric cell division. Analysis of the dynamic expression of the F-actin binding protein Anillin - an essential regulator of cleavage furrow progression and midbody formation in proliferating cells - suggests a link between apical asymmetric inheritance at cytokinesis and daughter cell fate. Anillin knockdown favours symmetric versus asymmetric divisions and concomitantly leads to symmetric F-actin distribution. This study thus provides in vivo evidence for a link between Anillin regulation of cytokinesis progression and intrinsic asymmetry of cell division, essential for the maintenance of the progenitor pool.

233. Characterization of the highly conserved m2de1 non-coding element associated with the *Meis2* gene. ***Tyler J. Ferrara**[1], Cody Barrett[2], Kyle Nelson[3], Ted Zerucha[1]*. 1) Biology, Appalachian State University, Boone, NC; 2) University of North Carolina at Chapel Hill School of Dentistry, Chapel Hill, NC, USA; 3) Wake Forest University Graduate School, Molecular and Cellular Biosciences, Winston-Salem, NC.
The Meis2 gene encodes a homeodomain containing protein that acts as a Hox cofactor that regulates development in vertebrate embryos. Meis2 is also a member of the TALE superclass, or three amino acid loop extension, which is a subset of homeodomain proteins that is characterized by an extra three amino acids between two of the alpha helices of the homeodomain. We have identified four highly conserved noncoding elements associated with the vertebrate Meis2 gene and named them m2de1-4 (for Meis2 downstream element). While m2de2-4have to date only been found in land vertebrates, m2de1 is also found in teleosts including zebrafish. The m2de1 sequence is approximately 450bp in length and its sequence and relative position to Meis2 (meis2a in zebrafish) is highly conserved amongst all vertebrates. Using the Tol2 system we have generated transgenic zebrafish in which either the zebrafish element (dr-m2de1) or mouse element (mm-M2de1) have been able to direct reporter transgene expression the mid and hindbrain of developing embryos. The m2de1 sequence was recently described by another group (Parker et al. 2011), however the expression of the reporter transgene being driven by their element was more restricted than the expression that we have observed. Upon closer examination, we have determined that our m2de1 sequence is slightly larger than reported in this publication, suggesting the possibility that their sequence does not represent the full length element. Reference: Parker et al. (2011) Ancient Pbx-Hox signatures define hundreds of vertebrate developmental enhancers. BMC Genomics 12:637.

234. Characterization of the highly conserved m2de2 non-coding element associated with the *Meis2* gene. ***Hannah Hemingway Freundlich**[1], Kyle Nelson[2], Ted Zerucha[1]*. 1) Biology, Appalachian State University, Boone, NC; 2) Wake Forest University Graduate School, Molecular and Cellular Biosciences, Winston-Salem, NC.
The Meis genes are highly conserved across species and crucial for embryogenesis. There are four known members of the Meis gene family in vertebrates, Meis1-Meis4. Because of the genome duplication event that occurred in the teleost lineage following the divergence from the lineage that would give rise to land vertebrates, zebrafish have 2 copies of the Meis2 gene, meis2.1 and meis2.2, in contrast to the single Meis2 gene in tetrapods. The Zerucha lab has identified four highly conserved non coding elements (CNEs) in tetrapods that we hypothesize direct Meis2 expression. We have named these m2de1-4 (for Meis2 downstream element). To date only one of these has been identified in zebrafish. The purpose of this study is to characterize m2de2 using zebrafish as a model organism. Using the Tol2 system, expression constructs containing mouse m2de2 driving expression of EGFP through the cfos minimal promoter have been constructed and microinjected into zebrafish embryos at the single cell stage. Confocal microscopy was used to determine EGFP expression at different time points post-fertilization. Expression was observed in the specific neurons in the brain of the developing zebrafish embryos in a pattern consistent with that observed for the murine Meis2 gene.

235. Intracellular MuSK localization and kinase activity in synapse positioning. ***Katherine D. Gribble**, Hannah K. Bell, Michael Granato.*
Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA.
Coordinated body movements depend on the precise synaptic apposition of spinal motor neurons and their target muscles at a specialized zone in the center of muscle cells. We have previously shown that before motor axons contact their target muscles, Wnt ligands bind the Muscle Specific Kinase (MuSK) receptor tyrosine kinase expressed exclusively on skeletal muscle. Wnt binding initiates MuSK receptor trafficking from the cell surface into Rab11-positive recycling endosomes localized to the center of muscle cells. Here, MuSK acts through components of the planar cell polarity (PCP) signaling cascade to restrict extending motor axons to the center of muscle cells, thereby marking where future neuromuscular synapses will form. MuSK trafficking to recycling endosomes is critical for synapse positioning, yet

how MuSK exerts its function inside muscle cells is not well understood. To dissect how MuSK functions inside muscle cells, we first asked whether the intracellular domain (ICD) of MuSK is sufficient to localize to the cell center. Expression of the wild-type MuSK ICD, or even an 'activated' MuSK ICD failed to properly localize to the cell center. However, fusing the MuSK ICD to a portion of the Rab11 interacting protein FIP3 resulted in correct localization of MuSK ICD to the cell center. Thus, we have developed a strategy to 'force' MuSK ICD localization to the cell center without prior ligand activation. Using this approach, we are currently testing whether MuSK kinase function is required after its internalization, e.g. for its central localization and to organize downstream PCP components. Alternatively, after its internalization, MuSK might act in a kinase-independent manner, e.g. as a scaffold to nucleate organization of PCP components. We will present the results of ongoing experiments to dissect the intracellular function of MuSK.

236. Molecular mechanisms of axon branch spacing in the zebrafish somatosensory system. ***Donald P Julien***, *Fang Wang, Alvaro Sagasti.* Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA.
At early stages of development, the skin is innervated by multiple populations of somatosensory fibers that terminate in the epidermis as highly branched free nerve endings, enabling animals to detect different types of sensory stimuli (e.g. chemical, thermal, mechanical). Separation of these complex terminal arbors into discrete innervation territories is critical for the accurate localization of stimuli along the body surface; however, the developmental mechanisms that govern the appropriate spacing of somatosensory axon branches remain poorly understood. In the embryonic zebrafish, peripheral sensory axon arbors are repelled by branches of the same arbor (self-repulsion) and neighboring arbors (neighbor-repulsion or tiling). The rapid development and optical clarity of the embryonic zebrafish make it an ideal model for uncovering the molecular signaling mechanisms that underlie these interactions. The Down Syndrome Cell Adhesion Molecule (Dscam) family of immunoglobulin transmembrane proteins has been implicated in self-repulsion and tiling in the nervous system of both flies and mice. We hypothesize that Dscam family members may play an analogous role in organizing vertebrate somatosensory axon branches. In situ hybridization and bacterial artificial chromosome (BAC)-mediated transgenic labeling studies demonstrate that the zebrafish ortholog, Dscamb, is expressed in somatosensory neurons during development. To determine if Dscamb has a functional role in organizing somatosensory axon branches, we used targeted mutagenesis to generate fish lines containing null mutations in the Dscamb locus. Phenotypic analysis of these mutant lines will determine if Dscamb is required for self-repulsion and/or tiling of peripheral sensory axons. To identify additional genes involved in these processes, we used fluorescence-activated cell sorting (FACS) to purify somatosensory neurons for RNA-sequencing and expression profiling. Analysis of embryonic somatosensory expression profiles will be used to identify candidate genes for sensory axon branch spacing, providing a basis for further mechanistic investigations.

237. Tfap2a Regulates Development of Neurons in the Inner Ear. ***Husniye Kantarci***, *Bruce Riley.* Texas A&M University, College Station, TX.
In all vertebrates hearing and balance information is relayed via the neurons of VIIIth cranial ganglion, known as the Stato-Acoustic Ganglion (SAG). SAG neuroblasts originate from the inner ear, which comes from an ectodermal thickening called the otic placode. The otic placode rapidly develops into the otic vesicle, a fluid-filled hollow ovoid from which many specialized cell types like hair cells, support cells and neuroblasts arise. SAG neurons are formed by a highly regulated multi-step process that starts in the otic epithelium. In the first step, SAG neuroblasts are specified in a region of the otic vesicle abutting nascent sensory epithelia. Neuroblasts soon delaminate from the otic vesicle and migrate to a region between the inner ear and hindbrain where they continue to proliferate (transit-amplification). SAG precursors eventually differentiate to form mature neurons, extending axons and dendrites to interconnect hair cells with information-processing centers in the hindbrain . Specification of the neural precursors in the ear requires low levels of Fgf signaling and proneural gene *neurogenin1* expression, but the other key regulators of the SAG development is very poorly understood. We observed that aspects of SAG specification, delamination and transit-amplification show marked resemblance with mechanisms regulating early neural crest development. Using a reverse-genetics approach, we have recently discovered that Tfap2a is expressed in the neurogenic domain of the ear and has a profound stimulatory function during the specification and differentiation of neural precursors through modulation of Notch, Fgf and Bmp pathways. The effects of Tfap2a on otic neurogenesis reveal a previously uncharacterized role for this gene and a novel mechanism for the development of SAG neurons.

238. Identifying Signals Required for Neural Tube Closure. ***Lexy Kindt***, *Ngawang Gonsar, Jessica Clay, Jennifer Liang.* Biology Dept, University of Minnesota Duluth, Duluth, MN.
Anencephaly is a fatal human developmental defect in which the anterior neural tube remains open. When Nodal signaling is reduced, zebrafish display a disorder similar to anencephaly. Previous work from our laboratory suggests a model where Nodal signaling acts through induction of the head mesendoderm to promote neural tube closure. The mesendoderm then promotes adhesion between neural tube cells. Cell adhesion is required for the movements that bring the edges of the left and right folds together so that they fuse at the dorsal midline and close the neural tube. Consistent with a central role for mesendoderm, we found that Nodal signaling is required up to dome stage (4.3 hpf) for a closed neural tube, which is within the time frame when Nodal activity is required for mesendoderm, mesoderm, and endoderm induction. However, the actual signal required for interaction between the mesendoderm and the neural tube is still unknown. Through RNA-sequencing, we are working to determine the identity of this signal. SB505124 is a small molecule that blocks the Nodal specific receptors ALK 4, 5, and 7 and decreases mRNA levels of Nodal regulated genes. We find that when treated with SB505124 at oblong-sphere stage (4 hpf), zebrafish embryos always have an open neural tube. In contrast, embryos treated at 30% epiboly (4.7 hpf) always have a closed neural tube. We treated embryos at oblong sphere and then raised them to stages when the signal between the mesendoderm and developing neural tube is likely prominent, then isolated their RNA for analysis by RNA-seq. We anticipate that this screen will find candidate genes for the head mesendoderm signal that promotes neural tube closure, genes expressed in the developing neural tube that would be affected by loss of this signal, as well as genes involved in mesendoderm differentiation.

239. Identification and mechanistic studies of novel gluconeogenesis regulators for anti-diabetic drug repurposing using transgenic zebrafish Pck1 reporters. ***Ji Dong K Bai***[1,2], *Youdong Wang*[2], *Philipp Gut*[3], *Xiao-Yan Wen*[1,2]. 1) Institute of Medical Sciences, Faculty of Medicine, University of Toronto, Ontario, Canada; 2) Zebrafish Centre for Advanced Drug Discovery, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada; 3) Department of Biochemistry and Biophysics, University of California-San Francisco, San Francisco, California, USA.
Background: Type 2 diabetes is a metabolic disorder estimated to affect 285 million adults worldwide and increases an individual's risk for cardiovascular diseases. Phosphoenolpyruvate carboxykinase (PEPCK) is an enzyme encoded by the Pck1 gene that catalyzes the rate-limiting step of gluconeogenesis. PEPCK is found to be transcriptionally regulated by glucose metabolism hormones such as glucagon and insulin. A transgenic Pck1:luc luminescent zebrafish reporter was developed for the purpose of identifying gluconeogenesis regulators. The goal of our study is to identify potential anti-diabetic drug leads and to develop methods for a fully automatic high-throughput drug screening using zebrafish embryos. Methods: In screening a FDA approved drug library, we exposed Tg(pck1:luc2) zebrafish larvae to small molecules at 4 days post fertilization (dpf), and measured the luminescence at 6 dpf. The amount of luminescence indicates the level of Pck1 expression. At St. Michael's Hospital, we have established an automated high-throughput screening platform for the screen. Results: Out of the 200 small compounds screened, we identified six molecules that affect luminescence, thereby potentially regulating PEPCK expression. Three of these molecules belong to the sulfonylurea class, which is known for their effects modulating glucose metabolism. The screen has also identified other classes of compounds (alpha adrenergic agonists) that may also modulate glucose metabolism. Conclusion: We identified several compounds that may regulate PEPCK expression in zebrafish. We are validating of the effectiveness of these compounds. Further directions include elucidating the mechanism of action of these compounds.

240. Pitt-Hopkins Syndrome: using the zebrafish to define mechanism and therapeutics. ***Bradley S. Carter***[1,2], *Alicia L. Blaker-Lee*[1], *Stephen J. Haggarty*[2,3], *Hazel L. Sive*[1,2,4]. 1) Whitehead Institute for Biomedical Research, Cambridge, MA; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Massachusetts General Hospital, Boston, MA; 4) MIT Department of Biology, Cambridge, MA.
Pitt-Hopkins Syndrome (PTHS) is a neurodevelopmental disorder associated with loss or mutation of one copy of the gene encoding the TCF4 transcription factor. The PTHS phenotype includes intellectual disability, epilepsy, craniofacial abnormality, and digestive disorders, including severe constipation. However, the connections between TCF4 action and normal body function are not well understood. Thus, it is not clear whether different *TCF4* mutations found in individual patients have different effects on the brain and other aspects of physiology, nor how to therapeutically correct the effects of TCF4 dysfunction in patients. We are using the zebrafish as a tool to address the connections between specific mutations in *TCF4* and PTHS-like phenotypes to form a personalized biorepository associating gene alleles and outcomes. In initial experiments, we have characterized both gain and loss of function phenotypes for *tcf4* in zebrafish and demonstrated specificity of the phenotypes in *TCF4* rescue assays. We are also using assays to examine *tcf4*-perturbed fish for seizure susceptibility and digestive function. In conjunction with iPS cell studies, these approaches will address phenotypes associated with patient-specific *TCF4* mutations and are essential for mechanistic understanding and therapeutic screening.

241. A transgenic zebrafish model for monitoring glucocorticoid receptor activity. *Randall G. Krug, Tanya L. Poshusta, Han B. Lee, Kimberly J. Skuster, Makayla R. Berg, Samantha L. Gardner,* ***Karl J. Clark***. Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN.
Gene regulation resulting from glucocorticoid receptor and glucocorticoid response element sequence interactions is a hallmark feature of the vertebrate stress response system. Imbalances in stress response signaling have been linked to socio-economically crippling neuropsychiatric disorders, and thus in vivo models are needed to help understand disease progression and management. Therefore, we developed a transgenic zebrafish reporter line with six glucocorticoid response element sequences used to promote expression of a short half-life green fluorescent protein (GFP) following glucocorticoid receptor activation. To characterize the ability of the reporter line to model glucocorticoid receptor signaling, transgenic larvae were either treated with exogenous glucocorticoid receptor ligands, or exposed to stressors including drugs of abuse or hyperosmotic conditions. The changes in GFP expression relative to control fish were assessed using both qRT-PCR and high-resolution imaging. Herein, we show that chronic and acute glucocorticoid treatment causes transgene activation in numerous tissues including the brain, and provides a single cell resolution in the effected regions. The specificity of these responses is demonstrated using the partial agonist mifepristone and mutation of the glucocorticoid receptor with transcription activator-like effector nucleases. Importantly, the reporter line also modeled the dynamics of endogenous stress response signaling, including the increased production of the glucocorticoid cortisol following exposure to stress and fluctuations of basal cortisol concentrations with the circadian rhythm. Collectively, these results characterize our newly developed reporter line for elucidating modifiers of stress response signaling, which may provide insights to the neuronal mechanisms underlying neuropsychiatric disorders.

242. Utilizing the larval zebrafish to study interactions between ethanol, dietary polyunsaturated fatty acids and hepatic lipid metabolism: Modeling the effect of diet on acute alcohol induced liver injury. *Vanessa Quinlivan-Repasi*[2], ***Steven Farber***[1,2]. 1) Embryology, Carnegie Institution for Science, Baltimore, MD; 2) Biology, Johns Hopkins University, Baltimore, MD.
Cirrhosis is advanced fibrosis of the liver that often leads to liver failure and can progress to hepatocellular carcinoma. When associated with metabolic syndrome or alcoholism, cirrhosis is typically preceded first by fatty liver disease (the accumulation of abnormally high levels of triglyceride in hepatocytes, abbreviated FLD) and then by steatohepatitis (chronic inflammation of the liver), both of which are reversible conditions. FLD is highly prevelent and correlates strongly with alcohol abuse, obesity and insulin resistance. Cirrhosis is thought to develop through chronic over-activation of the hepatic injury response. Although the role of alcohol over consumption alone in determining the progression of liver disease has been studied extensively, the contributions of fat stored in hepatocytes and consumed in the diet to the hepatic injury response are not understood. We hypothesize that dietary polyunsaturated fatty acids (PUFAs), already known to protect against lipotoxicity-induced cell death in the heart and to attenuate inflammation, will protect against steatohepatitis by

mitigating the inflammatory effects of alcohol. Larval zebrafish will serve as our model for investigation into how the presence of alcohol affects the metabolism of dietary lipids and the role of diet composition in alcoholic liver injury. To this end, we have optimized an ethanol treatment procedure that results in a significant increase in hepatic lipid content without affecting feeding behavior. We have also developed HPLC methods that will be used to analyze whole-body triglyceride and phospholipid composition of zebrafish larvae treated with ethanol and given a range of diets with varying fatty acid content. Liver injury will be assayed using qRT-PCR and in situ hybridization for inflammatory cytokines, immunostaining for fibrosis, and live imaging of the cell populations involved in the hepatic injury response in several fluorescent transgenic zebrafish lines.

243. Characterization of seizure-like behavior of free swimming zebrafish larvae submited to hyperthermia and pentylenetetrazole seizure-inducing models. ***M.C. Gonsales**, G.P. Gabriel, I. Lopes-Cendes, C.V. Maurer-Morelli.* Department of Medical Genetics, University of Campinas, Campinas, Brazil.
PURPOSE The purpose of this study is to characterize the seizure-like behavior of free swimming zebrafish larvae of 3 and 5 dpf in two seizure-inducing models: hyperthermia and pentylenetetrazole (PTZ). **METHODS** A pilot study was performed using larvae of two ages (3dpf and 5dpf) in three groups each: hyperthermia, PTZ and control. Five larvae were analyzed in each group for the age of 3dpf and 15 larvae in each group for the age of 5dpf. Each animal was observed during 10 minutes. Water temperature was maintained at 35°C for the hyperthermia group and at 25°C for the PTZ and control groups. Pentylenetetrazole 15mM was added to the water for the PTZ-treated group. **RESULTS** Larvae in the hyperthermia group presented hyperlocomotion, often moving towards the top and later displaying buoyancy dysregulation, marked by an inability to remain at a constant elevation. The number of seizure-like response was variable, with a few larvae presenting only increased natatory activity and others presenting several seizures, usually characterized by circling, spasms and tremor followed by ataxia. Seizure latency appears to be higher in 3dpf than in 5dpf larvae. All PTZ-treated larvae remained hypoactive during the first minutes of exposition. Seizures were characterized by rapid 'whirlpool-like' swimming, clonus-like convulsions and subsequent loss of posture. The number of seizures was similar in both ages. PTZ-treated larvae of 5dpf presented higher seizure latency than the ones with 3dpf. **CONCLUSION** Our preliminary results illustrate characteristic behaviors evoked by two different seizure-inducing models. There appear to be less sensibility to temperature-induced seizures in younger larvae, while 3dpf larvae submitted to PTZ presented seizure-like behaviors earlier than those of 5dpf. However, we still cannot confirm whether latency correlates with age in these cases. Since the studies using zebrafish to elucidate the basis of seizure generation are still relatively scarce, our study may provide new insights into the mechanisms underlying seizures. Supported by CEPID-FAPESP.

244. Methylmercury detoxification mechanism by the exosomal secretory pathway in zebrafish embryos. ***S. Imamura**[1], T. Yabu[2], T. Hara[1], T. Suzuki[1], K. Ishihara[1], Y. Yamashita[1], M. Yamashita[1].* 1) Natl Res Inst Fish Sci, Yokohama, Japan; 2) Nihon Univ, Fujisawa, Japan.
Dietary intake of selenium was suggested to reduce methylmercury (MeHg) toxicity in the 1970s. Here, we report the molecular mechanisms of MeHg detoxification by the novel selenium-containing imidazole compound, 2-selenyl-trimethyl-histidine, selenoneine (Yamashita and Yamashita, JBC, 285, 18134-18138, 2010). This compound has a strong radical scavenging activity, and is incorporated by a specific transporter, organic cations/carnitine transporter-1 (OCTN1). We characterized that both selenoneine and OCTN1 mediated MeHg detoxification by the exosomal secretory pathway in zebrafish embryos. The embryos at 8 h post fertilization were microinjected with MeHg-Cys (0.2 ng Hg/embryo) into yolk sac, and cultured in embryonic water for 24 h. The secreted exosomes released into rearing water from the embryos were collected by ultracentrifugation at 100,000 x g for 3 h, and their mercury and selenium contents and protein components were examined. The exosomes released in rearing water contained exosomal marker CD63, endosome marker Rab5, lysosomal and autophagic proteins (MAP1-LC3B, cathepsin L, exosomal serine protease), molecular chaperones (HSC70, CDC48), OCTN1 transporter, and ceramide, and the exosomal formation was enhanced by MeHg exposure, and such cellular function was accelerated in the presence of selenoneine. GFP-OCTN1 translocated from plasma membrane to granular in cytoplasm with MeHg-Cys (500 ng/ml in cultured media) in HEK293 cells, suggesting that OCTN1 transporter with the early endosome through an intracellular exocytosis involves MeHg detoxification. In addition, the treatment of embryos with H^+-ATPase inhibitor, bafilomycin A1, and the knockdown of ATG7, CDC48 or OCTN1 genes enhanced MeHg toxicity and caused severe apoptosis in central nervous system in the MeHg-injected embryos. Therefore, the exosomal secretory pathway triggered by endosomal sorting complexes required for transport (ESCRT) might involve in MeHg detoxification.

245. Gap Junctions Propagate Death Signals in the Adult Regenerating Retina. ***Kristin M. Ackerman**, Anthony Meena, David R. Hyde.* Biological Sciences, The University of Notre Dame, Notre Dame, IN.
Gap junctions are intercellular channels formed between adjacent cells to allow the translocation of ions, small metabolites, and second messengers from cell to cell. Gap junctions are composed of connexons, which are formed by six connexin (Cx) subunits. Approximately 40 Cx genes are predicted in the teleost genome and at least 16 were localized to the retina. Microarray data from our lab demonstrates differential expression of Cx genes during retinal regeneration, with the downregulation of Cx43 and upregulation of Cx43.4. Data from a retinal ganglion cell model of ischemic injury, indicates that global blockade of gap junctions via the pharmacological inhibitor, Meclofenamic acid (MFA), prevents widespread RGC cell death. To determine if gap junctions propagate signals during light-induced photoreceptor cell death, adult zebrafish were intraperitoneally injected with 1 mM MFA prior to constant light damage and every 12 hours during the damage paradigm. At 16 hours of light damage, the peak of photoreceptor cell death, cone photoreceptors in the MFA-treated retinas were healthy, which was in direct contrast to the light-damaged control retinas that displayed large numbers of pyknotic nuclei. The significant increase in the number of healthy cones in the MFA-treated retinas relative to the 16 hour light-damaged retinas correlated with a significant decrease in the number of TUNEL-positive cells in the MFA-treated retina. To determine which photoreceptor cell types were protected from cell death, control and MFA-treated retinas immunolabeled for cone opsins displayed a significantly higher number of green cones in the MFA-injected retinas compared to the 16 hour light-damaged retinas. Our laboratory previously demonstrated that a threshold

of damage was necessary to induce Müller glia derived proliferation. Despite the decrease in cell death, the MFA-treated retinas still displayed INL proliferation, although the number of PCNA-positive cells was significantly reduced relative to 36 hour light-damaged control retinas. These data indicate that widespread light-induced photoreceptor cell death signals are mediated by gap junctions and are required for Müller glia to mount a proper proliferation.

246. Calcineurin regulates coordinated outgrowth of regenerating zebrafish fins. *Satu Kujawski[1,5], Weilin Lin[2], Florian Kitte[1], Mandy Boermel[3], Steffen Fuchs[4], Guruschandar Arulmozhivarman[1], Sebastian Vogt[1,5], Denise Theil[1], Yixin Zhang[2],* ***Christopher L. Antos***[1]. 1) DFG-Center for Regenerative Therapies Dresden, Technische Universitaet Dresden, Dresden, Saxony, Germany; 2) B CUBE, Center for Molecular Bioengineering, Technische Universitaet Dresden, Arnoldstrasse 18, 01307 Dresden, Germany; 3) Institute of Biochemistry, Swiss Federal Institute of Technology Zürich, CH-8093 Zürich, Switzerland; 4) Universität Würzburg, 97072 Würzburg, Germany; 5) Max-Planck Institute for Cell and Molecular Biology, Pfotenhauerstrasse 108, 01307 Dresden, Germany.
Zebrafish develop and regenerate organs and appendages in a proportionally coordinated manner to the same dimensions as the original structures. Coordinated proportional appendage formation involves controlled regulation between allometric and isometric tissue growth. It is unclear what executes this control. We show that inhibition of the phosphatase calcineurin results in continued allometric regenerative outgrowth of regenerating fins beyond their original dimensions and continued transcription of regeneration-associated genes. Furthermore, calcineurin activity measurements are low when the rate of blastema outgrowth is highest and its activity increases as the regeneration rate decreases. Growth rate measurements of regenerating fins and morphometric analysis of proximodistal asymmetry of regenerated fins indicate that calcineurin inhibition shifts fin regeneration from a distal isometric growth program to an allometric proximal program. This shift is associated with the promotion of retinoic acid signaling, a signal transduction mechanism associated with proximal positional identity. In summary, we identified a calcineurin-mediated mechanism that operates as a molecular switch between distal isometric growth and proximal allometric growth.

247. Esco2 regulates *cx43* expression during skeletal regeneration in the zebrafish fin. ***Rajeswari Banerji****, Diane M. Jones, M. Kathryn Iovine, Robert V. Skibbens.* Department of Biological Sciences, Lehigh University, Bethlehem, PA.
Roberts syndrome (RBS) is a rare genetic disorder that is characterized by craniofacial abnormalities, limb malformation, syndactyly, cleft palate, corneal clouding and often severe mental retardation. RBS arises from mutations in *ESCO2* which encodes for an acetyltransferase that modifies the cohesin subunit SMC3 and is required for the establishment of sister chromatid cohesion during S phase of cell division. Cohesion is required to hold sister chromatids together until they separate during mitosis. Here, we use the zebrafish regenerating fin to reveal the role of *esco2* in the skeleton. We utilize morpholino-mediated gene knockdown of *esco2* and evaluate skeletal growth and patterning. The results show that *esco2* is upregulated during fin regeneration and specifically within the blastema, which is central to cell proliferation and cell signaling. Knockdown of *esco2* causes reduced regenerate length, segment length and cell proliferation. Interestingly, theses phenotypes mimic the phenotypes observed in *cx43* mutants (i.e. *short fin*, *sof* ^{b123}) and *cx43*-knockdown. Indeed, *esco2*-knockdown also causes reduced *cx43* expression. The *cx43* gene encodes the gap junction connexin subunit required for direct cell-cell communication. *CX43* mutations in humans results in oculodentodigital dysplasia (ODDD) - a developmental defect that includes craniofacial dysmorphia and syndactyly. These results conceptually link ODDD to cohesinopathies and provide the first evidence that *ESCO2* may play a transcriptional role critical for human development.

248. Characterization of *serpinh1b*/Hsp47, a Collagen Specific Molecular Chaperone Involved in Bone Growth. ***Joyita Bhadra****, Amanda M. Stillwell, M. Kathryn Iovine.* Biological Sciences, Lehigh University, Bethlehem, PA.
Skeletal morphogenesis is a process by which bones achieve their correct shape and size and joints are positioned appropriately. We use the regenerating caudal fin of zebrafish to study this process. Our examination of the fin length mutant *short fin* (*sof*) has revealed that the gap junction protein Cx43 is involved in skeletal morphogenesis by promoting cell proliferation and inhibiting joint formation, thereby coordinating skeletal growth and patterning. In order to understand how exchange of small molecules through gap junctions regulates joint development, we identified candidate downstream effectors of *cx43* through a microarray analysis. One of the genes validated from this microarray is *serpinh1b*. The gene *serpinh1b* codes for a protein called Hsp47, a molecular chaperone present in the ER which is responsible for proper folding of pro-collagen molecules. Recessive mutations in *SERPINH1* cause Osteogenesis imperfecta (brittle bone disease) in humans. The goal of this project is to characterize the function of *serpinh1b*/Hsp47 during skeletal morphogenesis. Knockdown of Hsp47 in the regenerating fin recapitulates the *sof* phenotypes of reduced fin length, reduced segment length and reduced level of cell proliferation. Whether Hsp47 regulates cell proliferation genes to promote growth or whether it plays a protective role against apoptosis is still not known. Current analyses are focused on distinguishing these possibilities through examination of markers for cell proliferation (i.e., *msxb*, *msxc* and *mps1*) and apoptosis (active *caspase-3*). Since Hsp47 is a molecular chaperone for collagens, an independent line of inquiry is to identify the effect of Hsp47 knockdown on collagen folding and collagen integrity in the fin skeleton. Together these findings will provide important insights into the role of Hsp47 in the skeleton, and into cellular functions regulated by Cx43.

249. A divergent role for anterior gradient protein 2 during larval and adult zebrafish fin regeneration. ***Elizabeth Brochu****, Ian Wanner, Sandra Rieger.* MDI Biological Lab, Salisbury Cove, ME.
Zebrafish and urodele amphibians, such as newts and axolotl, possess the remarkable capacity to regenerate their appendages as larvae and adults. Limb regeneration in salamander species requires the amputated limb stump to be re-innervated by nerves. When newt limbs are denervated prior to amputation, they lose their regenerative capacity due to the absence of newt Anterior gradient protein 2 (nAG) expression in the nerve and gland cells of the wound epidermis. As a consequence, the resulting blastema, a mound of dedifferentiating cells that gives rise to newly outgrowing limb structures, regresses. The family of anterior gradient proteins is highly conserved among vertebrates; yet, humans and most other vertebrates do not regenerate their limbs. Identifying the mechanisms underlying Agr2-dependent

limb regeneration in regenerating species could therefore hold the key to human limb regeneration in the future. The focus of this study was to investigate the role of Anterior gradient protein 2 (Agr2) in zebrafish appendage regeneration. Unlike newts, our results demonstrate that Agr2 knockdown with oligonucleotide morpholinos (MOs) enhances developmental fin growth and also fin regeneration in larval zebrafish, whereas adult fin regeneration is compromised after MO electroporation into the adult fin blastema. We further identified that proliferation cannot account for the increased fin growth in the larvae but that increased cell movement and fin expansion may be an underlying mechanism. We are currently investigating changes in cell size as an additional factor. The increased fin growth in *agr2* knockdown larvae was abrogated when skin innervation by peripheral sensory axons was concomitantly prevented, suggesting that similar to newts, Agr2 is regulated by peripheral neurons. Based on our observations, we propose a divergent role for Agr2 during larval and adult fin regeneration. We will further analyze Agr2 binding proteins with mass spectrometry to compare differences in Agr2 regulation during fin regeneration between larval and adult zebrafish.

250. Regulation of Zebrafish Fin Regeneration by miR-21. ***Heather R Carlisle***, *Benjamin King, Ashley M Smith, Viravuth P Yin.* Davis Center for Regenerative Biology and Medicine, Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672, USA.
Appendage regeneration is defined by the transformation of quiescent, differentiated tissues into highly proliferative and regenerative blastemal cells. These dramatic cellular changes are accompanied with rapid modulation of gene expression, thus implicating miRNAs. Here we performed deep-sequencing studies to identify differentially expressed miRNAs in the regenerating adult caudal fins. Real-time qPCR analysis confirmed miR-21 as the most highly upregulated miRNA in response to injury. Extended qPCR studies show miR-21 is similarly upregulated in all five zebrafish fin appendages. In situ hybridization studies in zebrafish caudal fins reveal miR-21 expression is localized to the basal-epithelial tissue layer and distal blastemal cells. Experimental depletion of miR-21 levels with antisense oligonucleotides culminated in regenerative outgrowth and patterning defects in all fin types. Conversely, experimental increases in miR-21 expression with Tg(hsp70:miR-21)mdi1 showed enhanced regenerative growth. Furthermore, we show that miR-21 is essential to activate blastema formation and cell proliferation, through multiple signaling pathways. Using an integrated bioinformatics approach, we have identified fgf20A, bmp3, and timp3 as miR-21 putative target genes. Collectively, our studies implicate miR-21 as a key component of a miRNA genetic circuit for repair and regeneration of complex appendage tissues.

251. Hair cell and support cell maintenance in adult zebrafish lateral line. ***Ivan Cruz***, *David Raible.* Biological Structure, University of Washington, Seattle, WA.
The phenomenon of regeneration confers multicellular organisms with the ability to restore lost or damaged structures. The robustness and degree of such processes depend on numerous factors, including tissue location as well as age and species of the organism. Zebrafish have the remarkable ability to regenerate a wide range of tissues that mammals lack the ability to renew. One example is the lost ability to regenerate mechanosensory hair cells in the inner ear, which are required for hearing and balance. The zebrafish posses a subset of hair cells that are stereotypically located on the outside of the body into clusters called neuromasts that together form the lateral line system. In larval fish, hair cells rapidly regenerate from symmetrically dividing support cells after neomycin-induced hair cell death. However, much is still unknown about the cellular hierarchy that controls regeneration and maintenance of hair cells and support cells in zebrafish lateral line. Using various transgenic lines, we show that adult zebrafish can regenerate hair cells and support cells after multiple iterations of ototoxic insults. Within the adult neuromast there are support cells with low and high proliferative rates, as assessed by fluorescent protein retention and clearance, which produce hair cells at different rates. Also, there is continuous loss and replacement of hair cells under ambient conditions. Our results indicate that the lateral line system has an incredibly robust regenerative ability that is retained into adulthood, and that at least two different support cell populations exist to maintain tissue integrity.

252. Genetic Determinants of Positional Memory During Appendage Regeneration. ***MaryLynn FitzSimons***[1,2], *Heather Carlisle*[2], *Cong Tian*[1,2], *Viravuth Yin*[1,2]. 1) Graduate School of Biomedical Sciences and Engineering - University of Maine, Orono, ME; 2) Mount Desert Island Biological Laboratory, Salisbury Cove, ME.
In the United States alone, almost two million people live with limb loss. Amputees suffer from significantly lowered health status, as well as increased rates of mortality; therefore, it is critical to elucidate mechanisms that promote successful limb regeneration. While humans possess limited regenerative capacity, the zebrafish displays the remarkable ability to completely regenerate damaged or lost appendages; however the mechanisms governing this process are not completely understood. During appendage regeneration, an information gradient along appendage axes is believed to regulate positional memory - the meticulous and appropriate replacement of complex tissues. Because both microRNAs (miRNAs) and retinoic acid (RA) are recognized to regulate appendage regeneration, we examined the gradient expression of miRNAs and components of the RA signaling pathway in tissues isolated from three planes along the proximal-distal (P/D) axis in the zebrafish caudal fin. Initial microarray analysis identified miRNAs with significant changes in expression during regeneration. Among these, miR-21 revealed the most dramatic upregulation. qPCR studies demonstrated that expression of miR-21 increases in a gradient along the P/D axis in both uninjured and regenerate fins. In contrast, two components of the RA signaling pathway, RALDH-2 and RXR-aa, are downregulated along the same gradient. The inverse correlation between expression of miR-21 and RA pathway members suggests that miR-21 may play a role in modulating RA signaling. This hypothesis is supported by previous experiments, which demonstrated that the RA co-receptor RXR-D is a direct target of miR-21, and that zebrafish caudal fins treated with anti-miR-21 are characterized by proximalization of structures during regeneration, reflecting patterns observed with RA treatment. Our results suggest that miR-21 regulates positional memory through targeting key components of the RA signaling pathway. This study presents important preliminary data contributing to an understanding of successful limb regeneration.

253. Haploinsufficiency of Def Activates p53-Dependent TGFb Signalling and Causes Scar Formation after Partial Hepatectomy. *Z. Zhi*[1], ***C. Gao***[1], *J. Chen*[2], *J.W. Xiong*[3], *J.R. Peng*[1]. 1) College of animal science, ZJU, Hangzhou, Zhejiang, China; 2) College of Life Sciences, ZJU,Hangzhou,Zhejiang, China; 3) Institute of Molecular Medicine, Peking University, Beijing, China.
The metazoan liver exhibits a remarkable capacity to regenerate lost liver mass without leaving a scar following partial hepatectomy (PH). Whilst previous studies have identified components of several different signaling pathways that are essential for activation of hepatocyte proliferation during liver regeneration, the mechanisms that enable such regeneration to occur without accompanying scar formation remain poorly understood. Digestive-organ-expansion-factor (Def) is a nucleolar protein that has recently been shown to complex with calpain3 (Capn3) to mediate p53 degradation specifically in the nucleolus. Here we use the adult zebrafish liver, which can regenerate within two weeks following PH, as a new genetic model to study the role of the Def-Capn3-p53 pathway in liver regeneration. Firstly, we show that Def expression is up-regulated in the wild-type liver following amputation, and that the defhi+/429 heteroozygous mutant (def+/-) liver expresses only ~1/4 of the Def found in a WT liver. We then show that the expression of pro-inflammatory cytokines is up-regulated in the def+/- liver, which leads to distortion of the migration and the clearance of leukocytes after PH. Transforming growth factor b (TGFb) signalling is thus activated in the wound epidermis in def+/- due to a prolonged inflammatory response, which leads to fibrosis at the amputation site. Fibrotic scar formation in def+/- is blocked by the over-expression of Def, by the loss-of-function of p53, and by treatment with anti-inflammation drug dexamethasone or TGFb-signalling inhibitor SB431542. We finally show that the Def-CAPN3-p53 pathway suppresses fibrotic scar formation probably through the regulation of the expression of the pro-inflammatory factor, high-mobility group box 1. We conclude that the novel Def-Capn3-p53 nucleolar pathway functions specifically to prevent the formation of scar tissues at the amputation site of the normal post-PH liver.

254. A b-catenin 2/Sox2 signaling pathway regulates Müller glia proliferation in the regenerating zebrafish retina. ***Ryne A. Gorsuch***, *Clare E. Yarka, David R. Hyde.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
In response to light-induced photoreceptor apoptosis, the zebrafish retina undergoes a robust regenerative response. This response initiates with a subset of Müller glia (MG) dedifferentiating and dividing to produce a transiently amplifying neuronal progenitor cell (NPC) population. NPCs continue to proliferate and migrate from the inner nuclear layer to the outer nuclear layer, where they differentiate into new rod and cone photoreceptors. MG dedifferentiation is an essential, rate-limiting step in the regeneration program, but the molecular mechanisms that control this process are just starting to be elucidated. Sox2 is an established neural stem cell marker *in vivo*, making it an ideal candidate for MG reprogramming. Basal Sox2 expression is maintained in MG of the adult zebrafish retina, however, qPCR revealed that *sox2* expression significantly increased when MG dedifferentiate and begin proliferating. Morpholino-mediated knockdown of Sox2 prior to light-damage significantly reduced the number of proliferating MG compared to controls, suggesting Sox2 is required for MG to dedifferentiate and reenter the cell cycle. Furthermore, overexpression of Sox2 in undamaged retinas was sufficient to stimulate MG proliferation. Previous studies have shown that b-catenin regulates *sox2* expression during vertebrate retinal development, and Wnt signaling was implicated in zebrafish retinal regeneration. To determine if b-catenin regulates *sox2* expression in the regenerating retina, we knocked down both b-catenin paralaogs (*ctnnb1* and *ctnnb2*), and observed a similar reduction in MG proliferation, as well as decreased Sox2 expression in MG compared to control retinas. qPCR analysis revealed that *ctnnb2* expression increased prior to *sox2* and *ctnnb1*, suggesting b-catenin 2 may independently regulate *sox2* expression. Morpholino-medaited kockdown of each paralog individually confirmed that loss of b-catenin 2 prevented both MG proliferation and upregulation of Sox2 expression compared to control and *ctnnb1* morphant retinas. These data identify a b-catenin 2/Sox2 signaling pathway required for MG dedifferentiation and proliferation in the regenerating zebrafish retina.

255. Discerning the role of the HA-Hapln1a ECM in the regenerating fin skeleton. ***Jayalakshmi Govindan***, *M Kathryn Iovine.* Biological Sciences, Lehigh University, Bethlehem, PA.
The formation of skeleton with bones of various shapes and sizes, and their growth is a complex process involving a multitude of genes. Our lab has shown that *cx43*, a gap junction gene is required for skeletal growth (cell proliferation) and joint formation (patterning). Gap junctions made up of connexin subunits aid in the exchange of ions and second messengers between the adjacent cells. Mutations in zebrafish *cx43* produces the *short fin (sof*^{b123}*)* phenotype and is characterized by short fins, short bony fin ray segments and reduced cell proliferation. Recently we showed that the extracellular matrix (ECM) protein Hapln1a (Hyaluronan and Proteoglycan Link Protein 1a), functions downstream of Cx43. Knockdown of *hapln1a* in regenerating fins, resulted in reduced fin regenerate length, segment length, and cell proliferation, recapitulating all the phenotypes of *cx43* knockdown. Additionally, we found that *hapln1a* knockdown resulted in reduced hyaluronic acid (HA) levels. The function of Hapln1 is to "link" HA with proteins termed proteoglycans (PGs) like aggrecan and versican and provide stability to the ECM. Previous studies with other models have shown that, loss of Hapln1 protein or HA result in skeletal defects. However, very little is known about the functional role of the Hapln1a-based ECM, including HA during zebrafish fin regeneration. The current research aims at understanding and defining the role of HA/Hapln1a based ECM by manipulating the expression of HA. Preliminary data show that components of the HA pathway- namely, the HA synthesizing enzymes (Has- *has1* and *has2*) and HA degrading enzymes (Hyals- *hyal2*) are upregulated in regenerating fins as evaluated by in situ hybridization. To determine the specific roles of these enzymes regulating HA metabolism in regenerating fins, we utilized morpholinos to knockdown the proteins. Current data shows that knockdown of *has1* results in reduced regenerate length and knockdown of *has2* results in reduced regenerate and segment length revealing that HA plays an important role during fin skeleton regeneration. Further studies are aimed at understanding the effect of the knockdown on cell division and look at the levels of HA and the PGs in the knockdown fins.

256. Understanding the genetics of size and proportion. *J. Daane[1], J. Lanni[1,2], H. Boldt[1], S. Johnson[3], **M. Harris**[1]*. 1) Harvard Medical School, Children's Hospital Boston; 2) Wheaton College; 3) Washington University.
The formation and maintenance of scaling within an organ is essential for the physiological and mechanical function of our bodies. How proportion is regulated during development remains an enigma. Using forward genetics in the zebrafish, we have identified a class of mutants that lead to coordinated changes in organ size. Through analysis of one such mutant, *another long fin*, we have identified the K-channel, kcnk5b, as responsible for coordination of allometric fin growth (Perathoner 2014). Expression of gain-of-function mutations in *kcnk5b* during fin development is sufficient to cause changes in proportion. However, Kcnk5b is dispensable as null mutants exhibit wild type fin size. Recently, the suppression of calcineurin activity by FK506/cyclosporin was shown to cause an increase in size of regenerating fins suggesting that calcineurin may also play a role in size control (Kujawski 2014). We find that the growth profile of wild type fins after treatment with FK506 is similar to that of *alf/kcnk5b* mutant fins, suggesting regulation via similar processes. Importantly, we show that the action of FK506 is dependent on the presence of Kcnk5b protein indicating a primary role of that K channels activity in regulating calcineurin activity during coordinated growth. How K channels signal to regulate growth and size is not clear. We are investigating the molecular basis of FK506 action and whether the effect is indirect through modulation of ionic potential and its downstream signaling or direct through modulation of Kcnk5b function. Additionally, we have found that FK506/Kcnk5b-mediated overgrowth does not specifically change positional information of the fin, rather mediates an increased rate of growth. A significant finding is that regulation of size occurs normally in the absence of Knck5b -- even in the presence of FK506. Our data support a model in which Kcnk5b/FK506 control the rate of allometric and homeostatic growth, but do not specify the size limit of a structure or the positional information contained therein. Further analysis of fin-overgrowth mutants and conditional activation transgenics will extend these findings to understand how K channel signaling regulates coordinated growth and size.

257. Igf Signaling Is Required for Cardiomyocyte Proliferation during Zebrafish Heart Development and Regeneration. ***Ying Huang**[1,2], Michael Harrison[1], Arthela Osorio[1], Jieun Kim[1], Aaron Baugh[1], Cunming Duan[3], Henry Sucov[4,5], Ching-Ling Lien[1,4,5]*. 1) Heart Institute, The Saban Research Institute of Children's Hospital Los Angeles, Los Angeles, California; 2) Craniofacial Biology Graduate Program, Ostrow School of Dentistry, University of Southern California, Los Angeles, California; 3) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan; 4) Department of Surgery, Keck School of Medicine, University of Southern California, Los Angeles, California; 5) Department of Biochemistry & Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, California.
Unlike its mammalian counterpart, the adult zebrafish heart is able to fully regenerate after severe injury. One of the most important events during the regeneration process is cardiomyocyte proliferation, which results in the replacement of lost myocardium. Growth factors that induce cardiomyocyte proliferation during zebrafish heart regeneration remain to be identified. Signaling pathways important for heart development might be reutilized during heart regeneration. IGF2 was recently shown to be important for cardiomyocyte proliferation and heart growth during mid-gestation heart development in mice, although its role in heart regeneration is unknown. We found that expression of *igf2b* was upregulated during zebrafish heart regeneration. Following resection of the ventricle apex, *igf2b* expression was detected in the wound, endocardium and epicardium at a time that coincides with cardiomyocyte proliferation. Transgenic zebrafish embryos expressing a dominant negative form of Igf1 receptor (dn-Igf1r) had fewer cardiomyocytes and impaired heart development, as did embryos treated with an Igf1r inhibitor. Moreover, inhibition of Igf1r signaling blocked cardiomyocyte proliferation during heart development and regeneration. We found that Igf signaling is required for a subpopulation of cardiomyocytes marked by *gata4:EGFP* to contribute to the regenerating area. Our findings suggest that Igf signaling is important for heart development and myocardial regeneration in zebrafish.

258. The elucidation of cold responsive gene network and novel cis-regulatory elements in zebrafish. ***Peng Hu**[1], M. Liu[1], X. Li[2], L. Chen[1]*. 1) shanghai ocean university, shanghai, shanghai, China; 2) shanghai institutes for biological sciences, CAS, China.
How fishes orchestrate multi-tissue transcriptional changes to cope with cellular stresses induced by temperature fluctuations has attracted significant attentions in recent genomics studies. Although some cold-responsive genes have been identified, the systematic molecular network and the regulatory elements remain to be determined. Zebrafish *Danio rerio*, as a well annotated model fish, provides an opportunity to study the gene network and identification of regulatory elements under cold stresses. We conducted RNA-Seq to gain a glimpse in the multi-tissues transcriptional responses of zebrafish under increased levels of cold, from 28°C, 18°C to 9°C. Fuzzy k-means method was used to cluster transcriptional patterns across eight tissues. We characterized tissue-specific expressional patterns. Results indicated that different tissues showed distinct responses to cold stress. We found 231 genes were ubiquitously up-regulated in all tissues. Comparative transcriptomic analysis detected approximately 20% (39/231) of the commonly induced genes were the same as those found in common carp, suggested a universal cold-adaptive mechanisms in fishes. GO enrichment analysis showed that the common induced genes were involved in regulation of transcription, protein polymerization, response to cold and proteolysis. To elucidate the regulatory elements in response to cold, we conducted conserved motif analyses in the promoter region of the commonly induced genes, yielded two sequence motifs that are previously unrecognized to be cold responsive. Reporter gene assays with these two motifs *in vitro* revealed that the two motifs increased reporter-gene expression by 5-fold when the transfected cells were challenged at 18°C. In summary, by examining the cold-induced expression patterns, we identified distinctive functional features of tissue-specific response and universal stress response. Our analysis also found two novel regulatory elements responsive to temperature changes in zebrafish. The results shed new insights into the mechanism of gene regulatory control in cold temperatures in fishes.

259. Identification and Characterization of MicroRNAs in Zebrafish Sperm. ***Kuntong Jia***, *Meisheng Yi*. School of Marine Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China.
MicroRNAs (miRNAs) are involved in nearly every biological process examined to date. Mounting evidence shows that some sperm specific miRNAs play important roles in the regulation of spermatogenesis and germ cells development, but little is known of the exact identity, expression levels, and function of miRNA in sperm cells or their potential involvement in spermatogenesis and germ cells development. Here, we investigated the sperm miRNA profiles using solexa deep sequencing combined with bioinformatic analysis and stem-loop reverse transcription polymerase chain reaction using zebrafish as a model system. Deep sequencing of small RNAs yielded 12 million raw reads from zebrafish sperm. Analysis showed that the noncoding RNA of the sperm included tRNA, rRNA, snRNA, snoRNA and miRNA. By mapping to the zebrafish genome, we obtained 604 miRNA candidates from the sperm, and 204 of miRNA candidates were consistent with zebrafish miRNAs deposited in the miRBase database that could be grouped into 104 families, including zebrafish specific families, such as mir-731, mir-724, mir-725, mir-729 and mir-2185. Moreover, bioinformatic analyses revealed that the most abundant known miRNAs previously described to play roles during spermatogenesis and germ cells development, such as dre-miR-202-5p, dre-let-7a, dre-miR-122 and dre-miR-21. Furthermore, the ten most abundant known miRNAs in sperm were selected and further confirmed by real-time quantitative PCR for their expression characteristics in different types of tissue, our results showed specific and high expression of dre-miR-202-5p and dre-miR-21 in zebrafish sperm, implying the crucial roles of these two miRNAs during spermatogenesis. Together, we report the first characterization of the miRNA profiling in sperm from zebrafish. Importantly, we obtained a lot of miRNA candidates and identified two potentially spermatogenesis related miRNAs. The obtained sperm miRNA profiling will serve as valuable resources to systematically study spermatogenesis in zebrafish.

260. Knock-down of cilia associated *ift88* leads to olfactory dysfunction: Development of a new sensory cilia assay using genetically encoded calcium biosensors in zebrafish. ***Judith G.M. Bergboer***[1], *Cameron Wyatt*[2], *Emre Yaksi*[2], *Iain A. Drummond*[1]. 1) MGH, Nephrology Division, HMS, Dept of Genetics, Boston, MA; 2) Neuroelectronics Research Flanders, Leuven, Belgium.
Ciliopathies represent a range of complex human syndromes, which all have mutations in genes leading to abnormally formed and/or dysfunctional cilia. The goal of this project was to devise an in vivo, zebrafish-based method to measure sensory cilia function. We studied responses of the olfactory sensory neurons (OSNs) present in the olfactory epithelium (OE) after stimulation by different classes of odorants. In the OE of zebrafish four types of OSNs are known, one of these is ciliated. The ciliated OSNs are known to be activated upon stimulation by bile acids. To measure activity in the OSNs we used the genetically encoded fluorescent calcium biosensor GCaMP5 under the neuronal HuC promoter. For our analysis we focused on the activity in the OE. To study the effect of cilia malformation on olfactory functioning, we knocked down the intraflagellar transport gene, *ift88*, in the Tg(HuC:GCaMP5) transgenic line. Ift88 is a core component of the cilia intraflagellar transport complex and plays a critical role in cilia formation and maintenance. We tested the responses of the morpholino injected fish (N=5 or 6 per condition) to four different odorant stimuli: mixtures of bile acids, amino acids, or nucleotides, concentration of 10 mM, or 10 mM food odor in fish water. Our results show that in 2 - 3 dpf embryos, olfactory neurons show robust and reproducible calcium signals upon odorant stimulation. The number of responding OSNs towards any stimulus was reduced by over 50%; in the *ift88* morphants compared to the control morphants. In addition, in the 2 dpf *ift88* morphants the bile acid response in the OSNs was greatly reduced compared to the control morphants. Cluster analysis also revealed that in these fish, the activated OSNs were less able to discriminate between different odorant stimuli. In conclusion, we developed a novel method to study sensory cilia function in zebrafish. This method will be used to further explore mechanisms of sensory cilia function in vertebrate neurons and will provide a platform for in vivo screens for therapeutic compounds to treat ciliopathies.

261. Axon-Schwann cell interactions during peripheral nerve regeneration in zebrafish larvae. ***ML Ceci***[1], *C Mardones-Kurslovic*[1], *LE Valdivia*[1,2], *M Sánchez*[1], *ML Allende*[1]. 1) University of Chile, Santiago, CHILE; 2) Department of Cell and Developmental Biology, University College London.
Peripheral nerve injuries can severely affect the way that animals perceive signals from the surrounding environment. While damage to peripheral axons generally has a better outcome than injuries to central nervous system axons, it is currently unknown how neurons re-establish their target innervations to recover function after injury, and how accessory cells contribute to this task. Here we use a simple technique to create reproducible and localized injury in the posterior lateral line (pLL) nerve of zebrafish. Using single axon labeling by transient transgene expression, as well as transplantation of glial precursor cells in zebrafish larvae, we individualize different components in this system and characterize their celular behaviors during the regenerative process. We show that innervation of lateral line hair cells in neuromasts during pLL nerve regeneration is a highly dynamic process with promiscuous target recognition. Furthermore, Schwann cells are required for directional extension and fasciculation of the regenerating nerve. We also provide evidence that these cells and regrowing axons are mutually dependant during early stages of nerve regeneration in the pLL. The role of ErbB signaling in this context is also explored. The accesibility of the pLL nerve and the availability of transgenic lines that label this structure and their synaptic targets provides an outstanding in vivo model to study the different events asociated with axonal extension, target reinervation and the complex cellular interactions between glial cells and injured axons during regeneration. Funding: FONDECYT 3120073 (MLC), 1110275 and FONDAP 15090007 (MLA).

262. Analyzing the transcriptome of zebrafish sensory hair cells by TU-tagging. ***Timothy Erickson***, *Teresa Nicolson*. Oregon Hearing Research Center and Vollum Institute, Oregon Health and Science University, Portland, OR.
Hair cells are the mechanoreceptors of the auditory, vestibular, and lateral-line (acoustico-lateralis) organs in vertebrates. Given their high degree of specialization, uncovering which genes are specifically expressed in hair cells may provide some insight into how these how these cells sense and transmit information on sound, gravity, and water movements. However, the complex anatomy of the acoustico-lateralis organs makes the bulk isolation of hair cell-specific RNA challenging. To address this problem, we have turned to thiouracil (TU)-

RNA tagging in live zebrafish followed by RNA sequencing to analyze the hair-cell transcriptome. TU-tagging is a recently developed technique that allows for thiouridine-labeling of actively transcribed RNA in a specific cell type by using the spatially-restricted expression of a uracil phosphoribosyltransferase (UPRT) enzyme together with the global application of its substrate, 4-thiouracil. TU-tagged RNA can be biotinylated in vitro and isolated from non-labeled RNA using streptavidin purification. Using this technique to study the hair-cell transcriptome, we found 144 genes whose expression in hair cells was significantly enriched >2-fold relative to the total nascent mRNA population. Subsequently, RNA in situ hybridization (ISH) confirmed that many of the highly enriched transcripts are present only in hair cells. Moreover, ISH revealed that some enriched transcripts are restricted to subsets of hair cells that are specialized for auditory, vestibular, or lateral-line function. By virtue of their highly specific expression patterns, these genes become prime candidates for reverse genetic knockout strategies in zebrafish. With this approach, we will gain a clearer understanding of the genetic components required for development and function of sensory hair cells.

263. Divergent regulation of late-stage rod versus cone differentiation. ***Jennifer Hocking***, *Mika Asai-Coakwell, Ordan Lehmann, Andrew Waskiewicz.* University of Alberta, Edmonton, Alberta, Canada.
Photoreceptors have unique morphologies optimized for interaction with photons and transmission of visual signals. While factors controlling photoreceptor identity are known, little is understood about late-stage photoreceptor maturation, the time when a functional cell is being created. We previously identified deleterious mutations in growth and differentiation factor 6 (*GDF6*) in four human patients with congenital or early-onset retinal dystrophy. In zebrafish mutant for *gdf6a*, photoreceptors are initially formed properly, but subsequent development is highly abnormal: cones have apical domains shortened by up to 55% at 14 dpf, while rods show an opposite phenotype, with outer segments at least doubled in length at 14 dpf. Notably, the photoreceptors appear healthy and do not degenerate, but the fish are blind. We conducted RNA-seq on eyes dissected from wildtype and *gdf6a* mutant fish at 5 dpf, which is the time point when the phenotype first appears. In confirmation of our immunohistochemical results, rod transcripts are on average 2.34 fold increased, while cone-specific transcripts are reduced 0.65 fold. Moreover, we discovered a number of transcriptional changes in genes that may function in photoreceptor specification, adhesion, and morphology. We propose that rod fate is 'emphasized', while cone fate is 'de-emphasized' in *gdf6a* mutants, and we will use this system to examine the factors controlling photoreceptor maturation. We are now investigating how *sine oculis* genes, Protocadherin 8, and Crumbs/Mosaic Eyes may function downstream of Gdf6a to direct the formation of a mature photoreceptor.

264. Plant derived alkaloids ameliorate aminoglycoside-induced hair cell death in the zebrafish lateral line. ***Matthew W Kruger***[1], *Allison B Coffin*[1,2]. 1) School of Biological Sciences, Washington State University, Vancouver, WA; 2) Integrative Physiology and Neuroscience, Washington State University, Vancouver, WA.
Thirty six million Americans report suffering from some degree of hearing loss, leading to social isolation and a loss of economic vitality. Hearing loss most commonly results from damage to the sensory hair cells in our inner ear that transduce auditory stimuli into neural responses. Hair cell death can occur due to noise trauma, genetic mutations, and exposure to certain medications (ototoxins), such as the aminoglycoside antibiotics neomycin and gentamicin. Plant derived natural compounds, such as those used in traditional Chinese medicine, offer a novel source of protective drugs to prevent aminoglycoside ototoxicity. The zebrafish lateral line is an excellent model for discovering new otoprotective drugs because its externally located hair cells are structurally and functionally similar to mammalian inner ear hair cells, and respond similarly to aminoglycoside insult. Using the zebrafish lateral line we conducted a chemical genetic screen for compounds that prevent aminoglycoside-induced hair cell death. The screen identified three natural compounds, E6 Berbamine (E6), Isotetrandrine (Iso), and Hernandezine (Her), that all confer protection from aminoglycoside ototoxicity. E6 and Iso both show robust protection against both neomycin and gentamicin. Using fluorescently-tagged gentamicin, we found that both E6 and Iso attenuate aminoglycoside uptake, suggesting that these compounds confer protection by preventing aminoglycoside entry into hair cells. However, all three compounds are known calcium modulators and may still aid in ameliorating hair cell death via intracellular mechanisms, as changes in intracellular calcium are associated with neomycin-induced hair cell death. E6, Iso, and Her are alkaloids found across several plant genera, including barberry and herbaceous perennial flowering plants, with root extracts currently used to treat symptoms such as heartburn, fever, and fatigue. Discovery of novel uses for plant derived natural compounds may allow for quick translation to clinical trials, offering therapeutic options for patients taking these lifesaving antibiotics.

265. A novel biosensor fish reporting the activity of Yap and Taz, the nuclear transducers of the Hippo pathway. ***Matteo Astone***[1], *Andrea Vettori*[1], *Enrico Moro*[2], *Elena Enzo*[2], *Sirio Dupont*[2], *Francesco Argenton*[1]. 1) Department of Biology, University of Padova, Padova, Italy; 2) Department of Biomedical Sciences, University of Padova, Padova, Italy.
The Hippo signaling pathway is an evolutionary conserved tumor-suppressor pathway, which controls animal organ size orchestrating cell proliferation, cell death and cell-fate decisions. Defects in components of the Hippo cascade lead to organ overgrowth and carcinogenesis. The pathway ultimately results in cytoplasmic retention of Yap and Taz, the transcriptional co-activators that interact with Tead transcription factors to promote cell proliferation and survival. Zebrafish transgenic lines reporting Yap/Taz activity have recently been established. Yap/Taz activity domains revealed by this reporter are restricted to a few embryonic tissues and organs, mainly the eye, heart, muscle of the trunk and epidermis (Miesfeld and Link, 2014). We developed a novel Yap/Taz reporter zebrafish transgenic line expressing nuclear mCherry under the control of a promoter displaying 3 Tead DNA binding sites. Several independent germline 3xTead:mCherry expressing founder fish were identified. All the stable transgenic fish shared a similar expression pattern, that was maintained in subsequent generations. During development, strong mCherry signal was observed in the eye, heart, muscle, epidermis, neural tube, floorplate, notochord, hypochord, vessels and intestine. The reporter is still active in adult fish, but a more accurate analysis is needed to fully define Yap/Taz activity domains. Knockdown and overexpression approaches were used to validate the reporter line. Injection of a morpholino targeting Yap mRNA reduced mCherry expression in the entire embryo, whereas mRNA injection of constitutively active form of Yap, Taz and Tead strongly increased the reporter signal. 3xTead:mCherry represents therefore bona fide Yap/Taz reporter. Together with the *in vivo*

characterization of Yap/Taz spatio-temporal activation, we are currently investigating their interconnections with other key signaling pathways, whose integrated activity with the Hippo pathway is emerging.

266. A heptad repeat in the cytosolic juxtamembrane in plexin A3 allows for homooligomerization. ***Rachael E Barton***[1], *Bryan W. Berger*[1,2], *M. Kathryn Iovine*[3]. 1) Department of Chemical Engineering, Lehigh University, Bethlehem, PA; 2) Program in Bioengineering, Lehigh University, Bethlehem, PA; 3) Department of Biological Sciences, Lehigh University, Bethlehem, PA.
Plexins, in conjunction with neuropilins (NRPs) and semaphorins (Semas), are proteins crucial for the regulation of axonal development, lymphangiogenesis, cancer cell proliferation, and zebrafish fin regeneration. Understanding plexin homomeric interactions and subsequent disruption may result in a novel cancer treatment. Previous research suggests that plexin clustering leads to activation, and while it is understood that extracellular semaphorin binding induces the intracellular signaling via Ras GTPase-activating proteins, little is understood about the role of the transmembrane and cytosolic juxtamembrane (CYTO) regions in signaling and oligomerization. Site-directed mutagenesis in the plexin A3 CYTO domain suggest a heptad repeat allows for clustering of the full-length receptor both alone and in the presence of NRP2 and Sema3F, as indicated by AraTM and bioluminescent energy transfer results. The integration of structural and cell-based measurements provide insight into the molecular mechanisms of how plexin-dependent signaling regulates tissue growth.

267. FGF signaling impairment by altered catabolism of glycosaminoglycans leads to developmental bone defects in a Mucopolysaccharidosis Type II fish model. ***Stefania Bellesso***[1], *Marika Salvalaio*[2], *Roberto Costa*[2], *Ilaria Zancan*[2], *Rosella Tomanin*[1], *Enrico Moro*[2]. 1) Department of Women's and Children's Health, University of Padova, Italy; 2) Department of Molecular Medicine, University of Padova, Italy.
FGF signaling is a key pathway strictly involved in many stages of ossification and gain of function mutations in many FGF pathway components have been associated with bone diseases like craniosynostosis and chondrodysplasia. The fine-tuning of the FGF signaling pathway is achieved at different levels, both intracellularly and by extracellular glycosaminoglycans (GAGs), which play a critical role in ligand concentration and receptor-binding. In this work, we show that deficiency of iduronate 2-sulfatase (IDS), which is an enzyme involved in GAGs catabolism, perturbs FGF signaling. The defect in IDS's activity causes a rare storage disease called Mucopolysaccharidosis Type II (MPSII; OMIM +309900). MPSII pathological manifestations are due to accumulated GAGs into lysosomes, which give rise to organelle disorders and cytotoxicity. Since skeletal abnormalities are one of the major disabling aspect of the disorder, we have created a zebrafish model for MPSII, using a morpholino-based knock down technology, to analyze how IDS functional alterations lead to the pathological skeletal phenotype. Morphant fish display some typical patient manifestations such as hepatomegaly, liver GAGs accumulation and defects in chondrogenesis and osteogenesis. Using the *in situ* hybridization technique, we have demonstrated that IDS knockdown affects the expression of key FGF signaling markers at early stages, before a clear lysosomal impairment is detectable. Moreover, our results show an altered expression of bone-related markers and bone ossification. Therefore, we have hypothesized that IDS deficiency affects FGF signaling during early stages of development, thus leading to an impaired expression of genes involved in bone development.

268. The novel membrane estrogen receptor GPER positively regulates liver growth during development. ***S. Chaturantabut***[1*], *C. Cutting*[2], *T. North*[3], *W. Goessling*[2]. 1) Department of Molecular and Cellular Biology, Harvard University; 2) Genetics, Brigham and Women's Hospital, Boston, MA; 3) Pathology, Beth Israel Deaconess Medical Center, Boston, MA.
Estrogen plays crucial roles in adult reproductive organ growth and size determination; however, the impact of estrogen on non-reproductive organs such as liver is not well understood. We have previously performed a chemical screen in zebrafish and identified estrogen as a regulator of liver development. Using estrogen receptor agonists/antagonists, we demonstrated a biphasic effect of estrogen regulation: at the hepatic progenitor stage (<72hpf) exogenous estrogen decreases liver size (93%, n= 48), whereas once differentiated hepatocytes are present (>96 hpf), estrogen increases liver size (75%, n= 60) as determined by in situ hybridization for the hepatocyte-specific marker liver fatty acid binding protein (lfabp). Estrogen elicits regulatory effects through classical estrogen receptors (ERs) and a novel membrane G-coupled estrogen receptor (GPER). Our data demonstrated that the later effect of estrogen on liver size is mediated by GPER; gper is expressed in the developing liver from 48hpf when hepatocytes start to differentiate. Exposure to a GPER agonist (G1) and antagonist (G15), during liver maturation (>96hpf) dramatically increased (57%, n= 21), and decreased (92%, n= 38), liver size respectively, while modulators of ERs activity had no effects. Additionally, G15 treatment blocked the positive growth effects of estrogen on the maturing liver, indicating that estrogen signals through GPER to increase liver size. Estrogen or G1 exposure enhanced cellular proliferation, as measured by BrdU incorporation, but had no effect on apoptosis (TUNEL staining). Morpholino knockdown of GPER also decreased liver size (46%, n= 28), confirming the chemical exposures. Treatment of estrogen on GPER morphants blocked the effects of estrogen on liver size (52%, n= 41). GPER morphants exhibited normal primitive endoderm and hepatoblast formation, but demonstrated defects in bile duct formation as determined by in situ hybridization for sox9b. Taken together, these data identify GPER as a novel regulator of liver formation and growth during zebrafish development.

269. Intracellular Regulation of Canonical Wnt Signaling During Zebrafish Embryogenesis. ***Z. Cui***, *X. Yang, G. Song, C. Liu, Q. Li.* Institute of Hydrobiology, CAS, Wuhan, China.
Canonical Wnt signaling is one of intracellular signaling pathways that play crucial roles in vertebrate embryonic development, cell fate determination and maintenance of adult tissue functions. Activation of this pathway is closely associated with cytoplasmic degradation and nuclear accumulation of b-catenin. Currently, many proteins are found to control the activity of canonical Wnt signaling through regulating the intracellular localization of b-catenin. Cav-1 is the principal component of plasma membrane caveolae that negatively regulates a number of cellular signaling events. We have previously found that Cav-1 is required for proper dorsoventral patterning in zebrafish. Wnt and BMP signals act coordinately to negatively control transcriptional expression of Cav-1 gene during embryonic development. Cav-1

overexpression disrupts the nuclear translocation of b-catenin through the interaction with its scaffolding domain and this interaction is necessary for the ventralizing activity of Cav-1. Thus, maternally expressed zebrafish Cav-1 regulates dorsoventral patterning by limiting nuclear translocation of active b-catenin. Additionally, we have demonstrated the functions of leucine zipper tumor suppressor 2 (Lzts2) in the embryogenesis of zebrafish. Abrogation of lzts2 expression by its specific morpholino enhanced gastrula convergence and extension (CE) movements. Mechanistically, Lzts2 regulates the migration of embryonic cells and dorsoventral patterning through its limitation of Wnt/b-catenin activity since it can physically interact with b-catenin-1 and -2 and transport them out of the nucleus. Therefore, Lzts2 regulates gastrula CE movements, dorsoventral patterning, and midline convergence and specification of organ precursors through interaction with and the export of nuclear b-catenins in zebrafish. Obviously, in-depth studies on mechanisms underlying the export and import of b-catenin would reveal the control of asymmetric and tissue-specific activation of canonical Wnt signaling during zebrafish early development and the activity of intracellular b-catenin in pathological contexts. Here, we'll introduce our novel findings about the nuclear import of b-catenin in zebrafish.

270. The Sox, Ets, and Runx transcription factor families regulate the expression activity of the R2 enhancer of col2a1a. ***Sonja Dabizljevic****, Peter Lyson, Daniel Brissette, Rodney Dale.* Biology Department, Loyola University, Chicago, IL.
Cartilage is an important tissue in vertebrates beginning in the early embryo where it lays down the scaffolding for the skeleton, and continuing through adulthood where it makes up joints and intervertebral disks. The major component of cartilage is a filamentous protein known as Collagen type II, alpha 1 (Col2a1). Mutations in col2a1 in humans can lead to multiple congenital disorders and the early onset of joint and retinal deterioration. Our laboratory is interested in understanding the conserved transcriptional regulation of this critical structural gene utilizing the zebrafish, Danio rerio. Understanding the genetic network upstream of zebrafish col2a1a could produce novel therapeutic targets. Previously, we identified a 310 bp regulatory region (R2) 1.7 kb upstream of the transcriptional start site that is critical for cartilage, ear, and notochord expression of the col2a1a gene in zebrafish. Utilizing transgenic zebrafish EGFP reporter analysis, we have now narrowed R2 down to 120 bp that can still reproduce the full col2a1a mRNA expression seen by in situ hybridization. Further characterization allowed us to identify a 60 bp sequence that specifies cartilage and ear but eliminates notochord expression. By employing comparative genomic analysis we have identified three highly conserved putative transcription factor binding sites for the transcription factor families of Ets, Runx, and Sox. Generation of targeted deletions of these transcription factor binding sites in our transgenic reporter results in loss of EGFP reporter activity, suggesting their necessity for col2a1a R2 activity. Overexpression of these transcription factors, via mRNA injection, into the previously published Tg(R2c2a1a:GFP) zebrafish line results in reporter activity as early as 50% epiboly, approximately 6 hours before normal reporter activity has been seen.

271. Identification of novel neural crest and melanocyte genes through molecular phenotyping followed by CRISPR/Cas9 functional validation. ***Christopher M. Dooley****, John C. Collins, Neha Wali, Ross N. W. Kettleborough, Ian Sealy, Richard J. White, Catherine Scahill, Zsofia Pusztai, Samantha Carruthers, Amanda Hall, Richard C. Clark, Richard Gibbons, Nicole Staudt, Isabel Brocal, Peter Clarke, Jorge Zamora, Derek L. Stemple, Elisabeth M. Busch-Nentwich.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.
The neural crest is a pluripotent, temporally restricted, cell population capable of differentiating into tissues across germ layer boundaries. The gene regulatory networks required for the induction and differentiation of neural crest cells have been investigated over a broad range of vertebrate species but still remain incomplete. It is now clear that certain neural crest derivatives, specifically those destined to become melanocytes, can be retained as undifferentiated precursors in adult zebrafish. Understanding the genetic networks and the regulatory dynamics required for an induced pluripotent state and the melanocyte linage's ability to remain undifferentiated is of keen interest. Previously, we have developed a high-throughput knockout and phenotyping pipeline. We have extended this process to assess transcriptional changes of knockouts in single embryos via Illumina sequencing. Using DeTCT (Differential Expression Transcript Counting Technique) we quantify differential abundance of polyA RNAs in individual wild-type versus mutant embryos. These molecular phenotypes deliver a detailed depiction of gene regulatory changes as a consequence of mutations, genomic modification, or small molecule treatments and are the controlling factors leading to morphological phenotypic outcomes. Using this approach we have begun morphological and molecular phenotyping of classical neural crest and melanocyte mutants as well as new ZMP mutants. The differentially expressed transcripts derived from these experiments provide novel candidates for functional validation and further loss of function analysis. We are able to target these candidate knockouts using both ENU derived alleles and the CRISPR /Cas9 system, and then feed them back into our phenotyping pipeline. In this way we have identified and functionally validated novel neural crest genes.

272. BMP heterodimer signaling in the developing vertebrate embryo. ***James A. Dutko****, Mary C. Mullins.* Cell & Developmental Biology, University of Pennsylvania, Philadelphia, PA.
Bone morphogenetic proteins (BMPs) are important biopharmaceuticals in the treatment of skeletal conditions and in applications of tissue engineering. In these clinical applications as in vertebrate development, BMPs provide differentiation and growth cues in a time and dose-dependent manner. The earliest function of BMPs is to pattern the embryonic dorsoventral (DV) axis. In the zebrafish gastrula, high BMP signaling levels arise ventrally, whereas BMP antagonists attenuate signaling dorsally. Here BMP signaling requires two ligands, Bmp2b and Bmp7a, functioning exclusively as a heterodimer, and their corresponding type I receptors, Alk3/6 and Alk8. Why BMP heterodimers function as the obligate ligand while BMP homodimers, although present, fail to signal is fundamental to the BMP signaling mechanism and relevant to biopharmaceutical applications. Furthermore, evidence supports the role of BMP heterodimers in at least three other developmental pathways in zebrafish. Our goal is to elucidate the mechanism for the obligate function of BMP heterodimers in DV patterning. One model for the obligatory function of BMP heterodimers is that BMP antagonists preferentially block BMP homodimers. To test this model, we depleted BMP antagonists in embryos devoid of BMP heterodimers. If antagonists block BMP homodimers, we expect BMP homodimers to signal when BMP antagonists are absent. Intriguingly, signaling was not observed in embryos lacking BMP

antagonists and BMP heterodimers, indicating that BMP antagonists do not preferentially block BMP homodimers *in vivo*. When overexpressed, however, Bmp2b homodimers can signal, but unexpectedly, they require Alk8, the Bmp7a-associated receptor. This suggests that BMP heterodimers prevail by virtue of assembling two different classes of type I receptor in a signaling complex. We examined the function of each receptor class within the signaling complex. Interestingly, embryos are patterned by unactivatable Alk3a receptors. In contrast, Alk8 kinase activity is required for patterning. The BMP signaling pathway is heavily studied yet heterodimer function is understudied and represents a significant gap in our knowledge of the BMP signaling mechanism, which we are elucidating here.

273. Blood Vessel Architecture Controls Junctional Dynamics During Endothelial Cell Division. ***Vahap Aydogan***, *Anna Lenard, Heinz-Georg Belting, Markus Affolter.* Cell Biology, University of Basel, Biozentrum, Basel, Switzerland.
Endothelial cell division is essential for growth and elaboration of the vascular network. The vasculature of the zebrafish trunk is characterized by the formation of intersegmental vessels (ISV), which are arranged in a metameric pattern and are connected by the dorsal longitudinal anastomotic vessel (DLAV). ISVs and DLAV are both made up by tubes of different cellular architecture, which are generated by distinct morphogenetic processes. Whereas multicellular vessels form their lumen by a chord hollowing process, unicellular tubes are hollowed out by membrane invagination (a.k.a. transcellular lumen formation). Here we have analyzed the junctional dynamics during endothelial cell division in the context of multi- and unicellular tube formation. We find that in multicellular tubes, endothelial cell division uses a junctional behavior similar to that recently observed in 2-dimensional epithelia (e.g. Drosophila epidermis). In these tubes, cell junctions are maintained and recruited into the novel cell contact of the daughter cells. Furthermore, the lumen is always maintained in this process. By contrast, in the unicellular tube context the lumen becomes constricted and is ultimately abrogated in the plane of cell division, where a cell junction will form de novo. We are now analyzing the cytoskeletal dynamics, which may drive these distinct cell behaviors.

274. Characterization of Pedf and its receptors in zebrafish. ***Spencer Evans***, *Jacek Topczewski.* Pediatrics/LCHCRC, Northwestern University, Feinberg School of Med., Chicago, IL.
Abnormal blood vessel growth is associated with multiple pathological conditions including tumor growth and age-related macular degeneration (AMD). AMD is the leading cause of blindness in older populations. Control of the process of new blood vessel formation, angiogenesis, is an attractive therapeutic strategy to prevent tumor growth and combat AMD. Pigment epithelium-derived factor (PEDF) has been identified as a potent inhibitor of angiogenesis. Patients with AMD have lower levels of PEDF expression. Therefore, restoring normal PEDF level may help in treatment of this condition. The mechanism by which PEDF inhibits angiogenesis is still being investigated. A small 34 amino acid peptide derived from PEDF is sufficient to exhibit antiangiogenic activity. This peptide binds two receptors, PEDF Receptor and LAMININ 1 Receptor (PEDFR and LAM1R, respectively) in mammals. Zebrafish are an interesting *in vivo* model for assessing angiogenic activity due to a high fecundity, transparency of embryos, and availability of transgenic lines to fluorescently label endothelial cells. However, Pedf and its two receptors have not yet been characterized in zebrafish. Here, we present identification of zebrafish orthologs of *pedf, pedfr* and *lam1r*. We have determined that they encode protein highly conserved with mammalian counterparts. We have established expression patterns of these genes at embryonic and early larva stages of development. In addition, we are testing whether ectopic expression of zebrafish or mammalian Pedf is able to inhibit zebrafish angiogenesis. Potential conservation of function would provide evidence that zebrafish can be used as an attractive platform for discovery of Pedf-mimicking drugs with antiangiogenic activity.

275. Cellular and Molecular Characterization of Intestinal Vessel Development. ***Michela Goi***, *Sarah Childs.* Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1 Canada.
Many congenital and acquired human diseases are accompanied by de novo pathological blood vessel formation, termed angiogenesis. Zebrafish is an ideal model in the study of blood vessel development using genetics to identify new genes in blood vessel formation. Exactly how angiogenesis occurs during development and in disease, particularly arterial sprouting, is an area of active research. In contrast, much less is known about how venous vessels develop. Indeed the genetic program that controls patterning, growth and differentiation of venous sprouting remains poorly characterized. The vessels that form a vascular plexus around the gut in order to provide blood supply to the developing digestive system represents a candidate angiogenic model to get an insight into the mechanisms that drive venous angiogenesis. We have devised a live imaging protocol to watch these vessels migrate in real time. We find that the features of the developing gut vasculature (subintestinal vein) do not resemble those of other known vascular systems and do not follow the typical rules of other angiogenic structures. This venous bed shows common developmental steps but a variable patterning among embryos instead of a specific control of the structure as seen for the intersegmental vessels in the trunk. We calculated properties such as the area of vessel coverage over time, average number of compartments and migration speed. Small molecule inhibition of the Vegf and Pdgf pathways inhibited formation of the internal vessels of the subintestinal vascular basket, while zebrafish mutants for the gene encoding the receptor PlexinD1 show an increased number of vessels compared to the wild-type embryos. Our results suggest the developing gut vasculature is a unique model to shed insight into novel mechanisms utilized in venous angiogenesis.

276. Apelin Signaling Promotes the Emergence of Lymphatic Endothelial Cells by Activating AKT during Vertebrate Development. ***J.D. Kim***, *Y. Kang, J. Kim, H. Kang, H. Park, W Dunworth, H. Chun, S.W. Jin.* Yale school of Medicine, New Haven, CT.
Rationale: Apelin (Apln) and its cognate receptor (Aplnr/Apj) are essential for diverse biological processes including early cardiac development, homeostasis, metabolism, and immune response as well as vascular development. Although Apln and its receptor Aplnr are known to preferentially express within developing lymphatic endothelial cells (LECs) in vertebrates, in vivo function of Apln signaling in lymphatic vessels remain largely unknown. **Objective**: We aim to delineate the functions of Apln signaling during lymphatic development using in vivo model system. **Methods and Results**: We delineated how Apln signaling affects lymphatic development. A suboptimal level

of Apln/Aplnr signaling activity in zebrafish embryos caused severe defects in developing lymphatic vessels without affecting blood vessels, indicating that the function of Apln signaling is critical for lymphatic development. In addition, abrogation of APLN/APJ signaling substantially attenuated migration of human LECs, indicating the evolutionarily conserved function of Apln/Apj signaling in LECs. We found that attenuation of Apln signaling significantly decreased the level of phospho-AKT, but not phospho-ERK1/2 in LECs. Moreover, the lymphatic abnormality caused by the reduction of Apln signaling activity was exacerbated by the concomitant partial inhibition of AKT signaling activity. Taken together, our data suggest AKT may function as a major downstream effector of Apln signaling in developing LECs. Furthermore, our analyses suggest a scenario where Apln signaling may converge with Vegfc/Vegfr3 signaling at the level of AKT to ensure its sustained activity, which is essential for lymphatic development. **Conclusions**: Apelin signaling provides a critical function in lymphatic development by promoting the activity of AKT in vertebrates.

277. The Zebrafish Sub-Intestinal Vein Requires Multiple Vegf Ligands and Receptors for Proper Patterning and Development. ***Andrew L Koenig***[1,2], *Saulius Sumanas*[1,2]. 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) University Of Cincinnati, College of Medicine, Cincinnati, OH.
Vascular development is a complex process that is tightly regulated through multiple pathways, including Vegf signaling. Major vessels develop in stereotypical patterns both through de novo vasculogenesis from precursor cells known as angioblasts, as well as angiogenic sprouting from existing vasculature. In zebrafish, the axial vessels - dorsal aorta (DA), and posterior cardinal vein (PCV) - form and circulation begins by approximately 24hpf. After development of the axial vessels the next major vessel to appear in the trunk is the sub-intestinal vein (SIV). This vessel, along with the supraintestinal artery that develops slightly later, vascularize the digestive system and are essential for the absorption of nutrients. The timeline of development and morphology of the SIV is well known, but the mechanism of its development has yet to be characterized. Here we demonstrate the requirement of multiple Vegf ligands and receptors for the formation of the sub-intestinal vein, as well as demonstrate a mechanism by which the SIV forms by migration of endothelial cells from the PCV that then coalesce to form the new vessel. Utilizing a small molecule Vegf receptor inhibitor and antisense morpholinos against different Vegf ligands and receptors, our results indicate that there is redundancy in Vegf signaling requirement in the development of the SIV. Complete Vegf signaling inhibition by a small molecule inhibitor prevents any formation of the SIV, however knockdown of individual receptors or ligands results in partial defects in SIV development. Knockdown of some individual receptors also demonstrate greater SIV development defects than combined knockdown of their respective ligands, suggesting redundancy in the pathway at the level of both ligand and receptor. These findings demonstrate a mechanism for development of major gut vasculature in zebrafish and a functional redundancy in Vegf signaling.

278. GDF6 act synergistically with VEGF to regulate the invasive and migratory properties of endothelial cells during angiogenic sprouting. ***Shlomo Krispin***, *Amber N Stratman, Daniel Castranova, Sofia A Pezoa, Brant M Weinstein.* Program in Genomics of Differentiation, NICHD,NIH, Bethesda, MD.
The complex body design of vertebrates requires efficient transport of gases, metabolites, nutrients, hormones and circulating cells between tissues and organs, all of which depend on proper assembly and function of the circulatory system. The formation of new blood vessels during development depends mainly on angiogenesis, the sprouting of new vascular tubes from preexisting vessels. Angiogenic sprouting is a complex cellular process in which endothelial cells (EC) loosen their cell-cell junction contacts, activate proteases that degrade the surrounding basement membrane and acquire extensively invasive and motile behaviors. Bone morphogenetic protein (BMP) signaling has been implicated in regulation of these morphogenetic behaviors in several developmental paradigms. However, the role of bmp signaling in angiogenesis remains unclear with different ligands and receptors demonstrating different and sometimes opposing effects on the angiogenic balance. We find that the BMP ligand growth differentiation factor 6 (GDF6) regulates endothelial sprouting in zebrafish and human EC by initiating an endothelial to mesenchymal (EndoMT)-like program. GDF6 up-regulates Snail2, N-cadherin, and Rock1, and down-regulates VE-cadherin, a molecular signature of an EndoMT-like program. We also find that GDF6 is acting through the bmp receptor 1b (BMPR1B) and bmp receptor 2 (BMPR2) complex. GDF6, BMPR1B or BMPR2A loss of function results in EC failure to become fully invasive and migratory. Our results demonstrate a specific role for the GDF6 ligand in regulation of angiogenic sprouting via activation of EndoMT.

279. Genome-wide Serine Protease Knockdowns in Zebrafish: Identification of Prostasin in Factor VII Activation Pathway. *G. Khandekar,* ***H. Sundaramoorthi****, P. Jagadeeswaran.* Biological Sciences, University of North Texas, Denton, TX.
Human factor VII is a vitamin K dependent serine protease zymogen that plays a pivotal role in the initiation of coagulation. Its activated form, factor VIIa, circulates at 1% of the levels of total factor VII. The mechanism of the initial generation of factor VIIa has been the subject of controversy and continues to be elusive. Hepsin has been suggested to activate factor VII, however, knockout experiments in mice did not support that hypothesis. However, we have shown that hepsin knockdown in zebrafish resulted in reduced factor VIIa levels, suggesting species specific activation of factor VII. Interestingly, the mechanism of activation of hepsin itself is still an enigma. Since we have recently introduced a novel, cost-effective, piggyback knockdown technology, we utilized its power and screened for genes involved in factor VII activation. We chose 181 genes that encode for proteins containing serine protease domains for targeted knockdowns. Using the piggyback technology, we performed knockdowns of the above genes in adult zebrafish. We then screened their plasma with a kinetic Prothrombin Time (kPT) assay to select only those genes involved in the extrinsic pathway of coagulation, which includes factor VII. Gene knockdowns that yielded a prolonged kPT were then tested for a reduction in factor VIIa levels. We found a novel prostasin gene (*zgc:92313*) that plays a role in factor VII generation. Based on this result we propose that prostasin, known to be activated by autocatalytically activated matriptase, may in fact activate hepsin. This would constitute a transmembrane serine protease cascade which then initiates the extrinsic coagulation cascade.

280. Cooperative sculpting of the dorsolateral facial skeleton by Pou3f3 and Jagged-Notch signaling. ***Lindsey Mork***, *Elizabeth Zuniga-Sanchez, Gage Crump*. Broad CIRM Center, University of Southern California, Los Angeles, CA.
The bones of the face derive from neural crest cells that populate the pharyngeal arches in the early embryo, with the lower jaw deriving from ventrally located arch cells and the ear attachment skeleton from dorsal arch cells. Ventral arch identity is imposed by the Endothelin1 (Edn1) and BMP signaling pathways, while much less is known about the signals that establish dorsal fate. We previously identified a key role for the dorsally expressed Notch ligand Jag1b in counteracting the ventral program and shaping the dorsal arch-derived skeleton in zebrafish. We now show that another dorsally expressed gene, *Pou3f3*, has a conserved role in dorsal skeletal development. Similar to murine *Pou3f3* mutants, zebrafish *pou3f3a; pou3f3b* TALEN-generated mutants show specific defects in both perichondral and intramembranous skeletal elements derived from the dorsal arches. Like *jag1b*, *pou3f3a* and *pou3f3b* are specifically expressed in the dorsal arches and repressed ventrally by Edn1 signaling. However, unlike Jag1b, Pou3f3a/b do not actively antagonize the Edn1-driven ventral program. Consistent with Jag1b and Pou3f3 acting in parallel for dorsal skeletal patterning, Jag1b and Pou3f3a/b are not required for each other's expression, and *jag1b* and *pou3f3a/b* mutants show different abnormalities in the same dorsal elements. Moreover, we observe ectopic expansion of the precartilaginous condensation marker *barx1* in the dorsal arches in *jag1b* but not *pou3f3a/b* mutants. This finding indicates that Jag1b-Notch signaling functions to prevent certain dorsal cells from contributing to cartilage, instead potentially guiding them toward osteoblast fate and ensuring the size disparity in the intramembranous bones formed in the dorsal vs. ventral arches. Pou3f3a/b, by contrast, act later to stimulate the growth of dorsal cartilage elements and the maintenance of dorsal osteoblasts. Finally, triple *jag1b; pou3f3a/b* mutants present an additive phenotype that combines the distinct malformations of the individual mutants. Our findings indicate that the dorsal arch-derived skeleton is sculpted by the cooperative action of multiple pathways functioning in parallel to control distinct spatiotemporal aspects of skeletal differentiation.

281. Examining the Roles of RNA Polymerase Subunits Polr1c and Polr1d in Treacher Collins Syndrome and Craniofacial Development. ***Kristin Watt***[1,2], *Annita Achilleos*[1], *Paul Trainor*[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.
Treacher Collins Syndrome (TCS) is a craniofacial birth defect characterized by deformities of the facial bones, ears and palate. Craniofacial anomalies typically arise from defects in neural crest cells (NCC), which are a migratory progenitor cell population that generates most of the cartilage and bone of the head and face. TCS is associated with mutations in *TCOF1*, *POLR1C*, and *POLR1D*. Mutations in *TCOF1* are known to disrupt ribosome biogenesis and NCC development; however, very little is known about the function of *POLR1C* and *POLR1D*. Polr1c and Polr1d are subunits of RNA Polymerases I and III, and it is surprising that mutations in these genes lead to a specific craniofacial phenotype. Interestingly, live imaging and alcian blue staining of *polr1c* and *polr1d* mutant zebrafish reveal craniofacial anomalies characteristic of human TCS. Furthermore, examination of *polr1c* and *polr1d* expression during embryonic development indicates that these genes are dynamically expressed and enriched in the craniofacial region. Markers of NCC development reveal a diminishment of migratory NCC. This is likely the result of elevated p53-dependent cell death within the neuropeithelium and NCC progenitor population, as evidenced by TUNEL staining and Western blot assays. We hypothesize that mutations in *polr1c* and *polr1d* disrupt production of rRNAs. Semi-quantitative RT-PCR reveals a diminished production of the 45S transcript in polr1c and polr1d mutant embryos. In a hypothetical model, diminished rRNA production leads to nucleolar stress and activation of Tp53. Consistent with this model, inhibition of *tp53* in *polr1c* and *polr1d* mutants is able to improve the phenotype of mutant embryos. These unique zebrafish models reveal the importance of *polr1c* and *polr1d* in craniofacial development and will further our understanding and the development of treatments for the prevention of TCS.

282. Zebrafish in the Classroom website. ***Jennifer Liang***. Biology, University of Minnesota Duluth, Duluth, MN.
The "Zebrafish in the Classroom" website (http://www.zfic.org) was created six years ago by a dedicated group of eleven students as part of an experimental undergraduate biology course. The original purpose of this site has been constant, to provide a place for sharing ideas, resources, and protocols for using zebrafish in undergraduate classrooms. Since that time, over 200 undergraduate students have created original content for the site. New sections include information on publishing opportunities for K-16 students, games (mostly Jeopardy style) that help students learn Mendelian genetics, and links to a wide array of published materials that promote the use of zebrafish in education. A major renovation of the site is planned during 2015. Goals for this renovation include: (1) creation of additional virtual experiments that open up experiments to students without access to zebrafish, (2) inclusion of movies that illustrate the dynamic nature of development, and (3) opening the site to outside submissions. Please stop by this poster to share what current parts of the site you find most valuable and how you would like to see the site evolve and grow.

283. Assessment of estrogenic pollution in the Roanoke River (Virginia) using embryonic zebrafish as a bioindicator. ***Seth D. Fortmann***, *Tyler W. Barnes, Garrett D. Schaperjahn, Christopher S. Lassiter*. Roanoke College, Salem, VA.
Numerous products of the twenty-first century, including pesticides, fungicides, and pharmaceuticals such as birth control pills, contain estrogenic compounds. These contaminates pollute waterways around the world leading to negative biological consequences. To investigate the presence of estrogenic pollution in the Roanoke River (Virginia) we use two different approaches. The first involves qPCR to quantify the relative amounts of mRNA for the genes *vitellogenin1* and *elongation factor 1 alpha (elfa)*. *Vitellogenin1* is an estrogen sensitive gene involved in female oviparous animals while *elfa* is a housekeeping gene involved in transcription. By comparing the qPCR results from different waterway samples to the standard curve we will be able to make an inference about the presence of estrogenic pollution. The second approach uses a line of zebrafish that fluoresce green in the presence of estrogen or estrogenic compounds. The intensity of this fluorescence is dependent upon the concentration of these estrogenic compounds thus measuring this intensity allows us to infer about the presence of estrogenic pollution. Preliminary results indicate the presence of estrogenic compounds in the river. When

combined, the results of these two approaches will provide us with an accurate estimate about the scope of estrogenic pollution in the Roanoke River.

284. 5-hydroxymethylcytosine in the zebrafish genome and the role of the Tet proteins. ***A. Kolano***[1], *K. Shanmuganandam*[1], *M. Wawrzyniak*[1], *M. Pastor*[2], *M. Wojciechowski*[1], *M. Bochtler*[1,2]. 1) IIMCB, 4 Ks Trojdena St., 02-109 Warsaw, Poland; 2) IBB, 5a Pawinskiego St., 02-106 Warsaw, Poland.
Epigenetic reprogramming involves remodeling of histones modifications and variants, changes in transcription profiles and DNA demethylation. It is essential for normal development of animals and plants. During the last few years DNA demethylation mechanisms have been extensively studied, and both passive and active DNA demethylation pathways have been proposed. The former depend on the replication of DNA, while the latter require enzymatic activities which remove 5-methylcytosines (5mCs) and replace them with cytosines. The best characterized active demethylation pathway involves an oxidation step from 5mCs to 5-hydroxymethylcytosines (5hmCs) driven by Ten-Eleven-Translocation dioxygenases (TET1, TET2 and TET3). During mammalian development the epigenome is reset twice - in the zygote and in primordial germ cells (PGC). It has been shown that in mammals TET3 is the key player in DNA demethylation of paternal epigenome in zygotes, while TET1 and TET2 are engaged in the DNA demethylation in PGCs. Recent data for lower vertebrates suggest that the biological functions of 5hmC and TETs might differ. Morpholino-mediated knockdown of TET3 in Xenopus causes a relatively “late” phenotype neural development. This raises the question regarding the evolution of epigenetic reprogramming in vertebrates. We chose zebrafish as a model to study the role of 5hmC and the Tet proteins in lower vertebrates. Our phylogenetic analysis indicates that all three mouse TET genes (TET1 - TET3) have orthologues in zebrafish. The expression pattern of Tet enzymes analyzed by qRT-PCR resembles the one observed in mouse - higher Tet3 mRNA level before zygotic genome activation (ZGA) and higher mRNA levels of Tet1 and Tet2 after ZGA. Using immunofluorescence labeling, dot-blot and glucosylation assay we can detect the enzymatic 5hmC, the product of Tet activity, in gDNA isolated from embryos at different stages of development (before and after ZGA). Finally, the non-specific dioxygenases inhibitor dimethyloxaloylglycine (DMOG) reduces the level of 5hmC and compromises the development of the zebrafish embryos.

285. Live imaging reveals a trophic role for innate immunity during tumour initiation. ***Derek W. Laux***, *Isabel Ribiero Bravo, Yi Feng.* MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh.
The development and progression of cancer involves complex interplay between transformed cell and the host’s environment. Until recently, it was not possible to live image the earliest interactions between host and oncogene-transformed cells during tumour initiation. Early work in the lab has made it possible to visualize a transformed cell at its inception, and has revealed that the transformed cell elicits an Trophic Inflammatory response, in which recruited leukocytes promote transformed cell growth, at least in part, through COX-2 mediated PGE2 production. We now have refined our model, and can induce cell transformation with precise temporal control in skin epithelium. We are using this model to unpick the signals that are critical in establishing the leukocyte Trophic Inflammatory Phenotype during tumour initiation. Using a transgenic NF-kB reporter we have observed heterogeneity in NF-kB activity levels in leukocytes that interact with transformed cells. Currently, we are creating a transgenic reporter using ECFP-p65, to better understand how NF-kB activation correlates with leukocyte trophic support. In addition, we are testing how inhibition of the NF-kB pathway affects leukocyte phenotype and whether it is critical in supplying trophic support. We are also developing a series of genetic reporters for candidate signals, which will allow us to live image whether these signals are involved in leukocyte activation/behaviour toward transformed cells. Our data suggests that PGE2 not only stimulates growth of transformed cells, but also modulates leukocyte phenotype. We therefore hypothesize that lipid metabolites including PGE2 might be critical in establishing the Trophic Inflammatory Phenotype in the innate immune cells during tumour initiation. Utilizing various pharmacological inhibitors and mutant lines, we are beginning to tease apart the relative contributions of lipid metabolites to leukocytes phenotype switch during tumour initiation.

286. zebrafish ddx39a regulates embryonic cell proliferation and development. *Jinrong Ma*[1], *Ian Scott*[2], ***Xin Lou***[1]. 1) Model Animal Research Center, Nanjing University, Nanjing, China; 2) Hospital for Sick Children, Toronto, Canada.
Normal embryonic development requires a fine regulation of cell proliferation, which also the one that are deregulated during tumor formation and metastasis. Key regulators in development are also known to act as tumor suppressors or tumor-promoting factors. RNA helicases from the DEAD-box family are found in almost all organisms and have important roles in RNA metabolism from RNA synthesis to RNA degradation. The detail function and mechanism about how these helicases engage in scenarios of animal development and cancer still remain to investigate. In previous gene trapping screen, we identified a zebrafish line carrying a null allele of ddx39a, a DEAD box RNA helicase. Ddx39a mutant embryo showed aberrant enhanced cell proliferation and apoptosis. We also observed abnormalities on multiple tissues including sensory neuron, skeleton and cardiac muscle and cartilage. These data suggested ddx39a plays important role on the regulation of embryonic cell proliferation and development of multiple organs/tissues.

287. Role of cytosolic phospholipase A2 (PLA2G4A) in the progression of the G0/G1 phase of the cell cycle. ***Said Movahedi Naini***, *Wenqing Yin, Joseph Bonventre.* Medicine Dept, Renal Div, BWH, Boston, MA.
Cytosolic phospholipase A2 (cPLA2) is a calcium sensitive enzyme involved in receptor mediated eicosanoid production. In this study, we use the zebrafish as a vertebrate model to investigate the role of cpla2 in the progression of the cell cycle. We report that there are two cpla2 genes in zebrafish. zcpla2a and zcpla2b are expressed in spatially restricted, mostly non overlapping domains during early development. The catalytic domains are conserved in both zebrafish cpla2 genes, and they both have comparable phospholipase activities. By loss of function and gain of function experiments using cpla2 specific MOs, we show that down-regulation of zcpla2 causes a neurodegenerative phenotype marked by shrinkage of brain and otic vesicles, and a curved-up body. This phenotype was accompanied by a decrease in cell proliferation and an increase in apoptosis rate in zebrafish morphants. By examining different cell cycle regulators, we

discovered an increase in p27kip1 and a decrease in cyclin D1 protein and mRNA levels in embryos in which cpla2 had been knocked down. This cell cycle defect in cpla2 deficient larvae precedes the morphological phenotype and enhanced apoptosis. thereby, we unravel a new mechanism of cell cycle regulation by cpla2 at G0/G1 phases, previously unknown.

288. Genetic Approaches to Identify Therapeutic Targets for Ewing Sarcoma. ***Joanie Neumann***, *Daniel Zmau, Stefanie Leacock, James Amatruda*. UT Southwestern Medical Center, Dallas, TX.
Ewing Sarcoma is a malignant bone and soft tissue tumor of children and young adults. Ewing sarcoma is caused by chromosomal translocations that create novel fusions of the *EWS* gene with an *ETS* family transcription factor (most commonly *FLI1*) and are believed to function as aberrant transcription factors. While chemotherapy can cure some patients, more than half of adolescents with Ewing sarcoma die of disease within five years. The cellular origin and mechanisms of EWS-FLI1 mediated tumorigenesis are not known, impeding the development of alternative therapies. To better understand Ewing sarcoma, we developed *in vivo* zebrafish models and showed that somatic expression of human EWS-FLI1 in zebrafish causes Ewing sarcoma (Leacock et al., DMM 2012). Given that human ESFTs have neuroectodermal features, we created a tissue specific ESFT model using the *mitfa* promoter to drive the expression of hEWS-FLI1 specifically in neural crest-derived cells. Strikingly, we found that EWS-FLI1 expression in melanocytes results in 1) increased number of early larval melanocytes in the head and 2) an almost a complete absence of pigment in the juveniles and adults, suggesting that EWS-FLI1 specifically interferes with the development and/or survival of melanocytes. Also, a subset of *tg(mitfa:hEWS-FLI1);p53*$^{-/-}$ fish develop tumors resembling Ewing sarcoma. To gain mechanistic insight into EWS-FLI1 mediated tumorigenesis, we are using the melanocyte phenotype as a foundation to conduct genetic and drug based chemical suppressor screens. We hypothesize that these genetic approaches will allow us to identify EWS-FLI1 effector genes. Furthermore, to understand the effects of EWS-FLI expression at the cellular level we are using a gene trap approach to conduct live cell imaging in zebrafish embryos. The gene trap system consisting of *eGFP-2A-EWS-FLI* allows us to visually track and quantify EWS-FLI1 positive cells by GFP fluorescence. We are using this system to characterize EWS-FLI1 expression, cell survival, and proliferation in GFP positive cells. Together, these zebrafish models will provide molecular understanding of Ewing sarcoma development and will foster the development of improved, targeted therapies.

289. Zebrafish as a Translational Model for Human Overgrowth Syndromes. ***David Peal***[1], *Jennifer Lanni*[1,2], *Jacob Daane*[1], *Felecia Cerrato*[1], *Brian Lebow*[1], *Matthew Harris*[1]. 1) Boston Children's Hospital; Harvard Medical School, MA; 2) Wheaton College, Norwood, MA.
Zebrafish is quickly becoming a key model for assessing gene function in the etiology of human disease. We use the zebrafish to investigate the genetic and developmental causes of diseases affecting the skeleton. Here we describe our work on an overgrowth disorder, macrodactyly, in which the digits of the hand or feet grow disproportionately large yet maintain normal patterning. Macrodactyly is thought to be due to somatic mutations arising during limb development. To identify the genetic changes causing this disorder, we screened a panel of growth related genes from affected and control tissue in patients. We identified common mutations among macrodactyly patients suggesting putative roles in overgrowth. All patients harbored gain-of-function mutations in the PIK3CA gene, suggesting a key role of this gene in causing the phenotype. However, identical mutations have been observed in a variety of cancer and overgrowth disorders, suggesting that this single mutation may not be sufficient to cause the coordinated overgrowth seen in macrodactyly. Using an over-expression assay in the zebrafish, we assessed the function of activated PIK3CA in regulating coordinated overgrowth of the adult fins. We find that activated PIK3CA is not sufficient to cause overgrowth in wild type strains; however, it can potentiate growth phenotypes observed in the longfin mutant background. These findings suggest that genetic modifiers of PI3KCA may be essential for coordinated overgrowth. To dissect mechanisms of its regulation, we are currently examining the modifying effect of genes known to alter fin allometry in zebrafish in their effect on PI3K-mediated overgrowth. Further, we are examining the role of the vascular system in coordinated overgrowth, as it undergoes expansion and dilation in both fin mutants and PI3KCA-related growth disorders and may provide unique insight into the pathology of the disease. Thus, although fins and limbs are quite dissimilar structures, the zebrafish provides a useful model to interrogate the regulation of coordinated limb growth and its dysregulation in human disease.

290. *esco2* mutant defective in sister chromatid cohesion demonstrates genomic instability *in vivo*. ***Stefanie M. Percival***[1], *Holly Thomas*[1], *Adam Amsterdam*[3], *Nancy Hopkins*[3], *Jacqueline Lees*[3], *H. Joseph Yost*[2], *John M. Parant*[1]. 1) Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT; 3) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.
Sister chromatid cohesion (SCC) is a mitotic process that is vital for ensuring genomic integrity and is most commonly studied in the context of cell culture. In 2005, it was found that a defect in SCC resulted in a severe developmental disorder, Roberts Syndrome. The causal gene was identified as ESCO2, one of two vertebrate establishment factor homologs. Establishment factors are essential and responsible for cohesion by acetylating cohesin, the ring complex holding sister chromatids together.
An *esco2* mutant was identified through a unique genetic screen to identify mutants with genomic instability that were rescued by loss of p53. *Esco2* mutant embryos display similar phenotypes observed in RBS. Dynamic, live cell imaging of mitotic divisions in our *esco2* mutant revealed extensive chromosome segregation defects, spindle rotation, and genomic instability. Chromosome spread analysis discovered that depletion of *esco2* results in high levels of aneuploidy and a complete loss of cohesion while *esco2+/-* exhibit a weakened cohesion phenotype suggesting dose dependent effects of *esco2*. We hypothesize that phenotypic variation in RBS patients reflects a gradient of cohesion defects mediated by the balance of a number cohesion factors. We are presently generating mutants in these factors to test this hypothesis.
Though RBS is not associated with a predisposition to cancer, a few cases of neoplastic processes have been documented. Surprisingly, our lab has observed enhanced tumorigenesis in *esco2+/-; p53+/-* fish compared to *esco2 +/+; p53+/-*. We predict that this is due to an *esco2*-

induced loss of heterozygosity for p53. Future directions of this project include investigating other SCC components for mitotic and cohesion defects as well as their role in tumorigenesis.

291. Somatic evolution in normal development and cancer. ***Elizabeth B Perry***, *Richard M White.* Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY.
All of the cells in the adult body of a multicellular organism are derived from a single cell. Errors in replication and damage to DNA that occur during development can result in an adult body that consists of a mosaic of genetically heterogeneous cell populations. Cancer has been described as a disease of abnormal somatic evolution in which DNA changes that occur in somatic cells can confer a fitness advantage allowing those mutants to rapidly proliferate and ultimately cause disease. Although cancers are most frequently diagnosed in older adults, the striking amount of genetic heterogeneity present in many human tumors is consistent with the hypothesis that initiating mutational events may occur many years prior to the appearance and diagnosis of cancer. The purpose of this project is to quantify the rates at which somatic mosaicism is generated in normal tissues and tumors, and to understand factors that influence those rates. We are developing a zebrafish model of melanoma to compare patterns of somatic mosaicism in cancer lineages and in healthy tissues sampled throughout the life of the organism. We have generated transgenic zebrafish with an inducible fluorescent system (ubi:loxP-GFP-loxP-mCherry) to track lineages of melanocytes in a melanoma-prone (p53-/-; mitf-BRAFV600E +/+;) and non-melanoma prone background (p53+/+; ;BRAF WT/WT). We are adapting a reduced-representation sequencing approach developed for evolutionary genetic studies called Restriction-Associated DNA sequencing, (RAD-seq) in order to sequence the zebrafish genome in regions flanking restriction cut sites. Our preliminary data show that we can identify mutations that occur within normal tissue and tumors. We use molecular phylogenetic techniques to reconstruct an evolutionary history among normal and tumor cell lineages and to infer the rates at which somatic mutations are generated during development in melanoma-prone and wild-type zebrafish. These preliminary data are relevant to broader questions of cancer evolution and whether somatic mosaicism among normal cell populations influences cancer development later in life.

292. Altered *sox9b* Expression in TCDD Mediated Embryonic Cardiotoxicity. ***Joseph Gawdzik***[1,2], *Felipe Burns*[1,2], *Matthew Russell*[1], *Jess Plavicki*[1], *Richard Peterson*[1], *Warren Heideman*[1]. 1) School of Pharmacy, University of Wisconsin, Madison WI; 2) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison WI.
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an environmentally persistent and toxic compound. Zebrafish embryos exposed to TCDD (1ng/mL) at 4 hours post fertilization (hpf) display cardiac malformations characterized by atrial elongation, ventricular compaction and pericardial edema by 72 hpf. Previous work has shown TCDD represses *SRY-box 9b (sox9b)* gene expression in the embryonic zebrafish heart. Genetic ablation of *sox9b* expression in zebrafish embryos partially recapitulates the TCDD cardiac phenotype, suggesting reduced *sox9b* expression is a step towards the cardiac malformations coincident with TCDD exposure. How *sox9b* is repressed in the embryonic heart and in which cardiac cell types this process is occurring is unknown. We hypothesized cis-regulatory elements 5' of *sox9b* are mediating the repression of *sox9b*. We created *Tg(sox9b:eGFP)* zebrafish expressing *eGFP* using a 5' portion of the *sox9b* promoter. We have identified the -2450 to -939 (relative to the transcriptional start site) expanse as being necessary for TCDD mediated repression in the heart. Using confocal microscopy we determined *Tg(-939/0sox9b:eGFP)* zebrafish retain cardiac eGFP signal in response to TCDD treatment (n>5), while *Tg(-2450/0sox9b:eGFP)* zebrafish do not (n>5). We hypothesized conserved cis-regulatory elements exist in this portion of the *sox9b* promoter. An *in silico* analysis identified putative transcription factor-binding sites in this region including five core Aryl Hydrocarbon Receptor response elements, two having high (>95%) matrix similarity as analyzed by MatInspector. Lastly, we hypothesized embryonic cardiomyocytes are sensitive to misregulated *sox9b* expression. We expressed *sox9b* using the cardiomyocyte specific *cardiac myosin light chain 2 (cmlc2)* promoter, *Tg(cmlc2:sox9b)*. Brightfield microscopy revealed *Tg(cmlc2:sox9b)* embryos with enlarged and unlooped heart chambers and pericardial edema (55%, n=13) by 96 hpf. This phenotype was absent (0%, n=13) in control *Tg(cmlc2:tRFP)* embryos. These findings shed light on where and how TCDD represses sox9b in the embryonic heart.

293. Chromatin Regulation of Cardiac Progenitor Cell Specification. ***Emily Irey***[1], *Alex Akerberg*[2], *Kryn Stankunas*[2]. 1) Department of Biology, University of Minnesota-Morris, Morris, MN; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR.
Transcription factors (TFs) are the most frequently mutated class of genes in congenital heart disease (CHD). Many of these TFs function early in development to specify a cardiogenic gene expression program in progenitor cells. However, little is known about how these TFs are themselves induced and how they function within chromatin landscapes to regulate transcription of their target genes. Various histone modifications are correlated with either transcriptional activation or repression. These covalent marks are added and removed by histone modifying enzymes, affecting transcriptional changes directed by cooperating TFs. Using zebrafish embryos, the Stankunas lab screened expression of histone modifiers during early heart development. One specific modifier showed strong heart expression and its morpholino knockdown resulted in severe cardiac defects. Interestingly, morphant embryos showed a decrease in Nkx2.5, one of the most commonly mutated TFs in CHD. In the present research, we sought to determine how the modifier activates Nkx2.5, either directly or by controlling expression of additional cardiogenic TFs that act upstream of Nkx2.5. We approached this question experimentally by asking whether morphant embryos would result in altered expression of Nkx2.5 regulators, reasoning that upstream factors would show altered signs of expression in the morphants. Thus, we hypothesized that when this histone modifier is knocked out, it will have adverse affects on possible upstream regulators of Nkx2.5, which would help account for the decreased expression we observed. We tested this prediction by analyzing expression differences of known Nkx2.5 regulators, including Gata4, Gata5, and phospho-SMADS, between control and morphant embryos by in situ hybridization and immunofluorescence. Our results showed no substantial expression changes, supporting the conclusion that Nkx2.5 is regulated directly by the covalent histone modifier.

294. Rtf1 is required for the formation of cardiac progenitor cells. ***Fei Lu***, *Adam Langenbacher, Jie Huang, Qingyu Li, Kevin Wang, Jau-Nian Chen*. Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles.
We have previously performed a forward genetic screen in zebrafish to dissect critical genetic pathways that direct cardiac development. From this screen, we discovered an essential role for the RNA polymerase II-associate factor 1 complex (PAF1C) in cardiogenesis. Zebrafish embryos deficient in ctr9, cdc73 and paf1, three PAF1C components, have reduced cardiac tissues, whereas loss of function of rtf1, another PAF1C component, eliminates the entire cardiac progenitor population. Overexpression of rtf1 promotes the expression of cardiogenic genes in developing zebrafish embryos without inducing markers of other cell lineages, and is sufficient to drive cardiogenic gene expression in cdc73/ctr9/paf1 triple knockdown embryos. These observations indicate that Rtf1 potentiates the production of cardiac progenitor cells in a PAF1C independent mechanism. Furthermore, chromatin immunoprecipitation assay shows that Rtf1 occupies the promoter region of multiple cardiac genes, suggesting that Rtf1 drives the formation of cardiac progenitor cells through regulating cardiac transcription machinery. Moreover, knocking down Rtf1 activity in mouse ES cells prevents these cells from differentiating into cardiomyocytes. These findings demonstrate a critical early role for Rtf1 in specifying cardiac lineage from the mesoderm and indicate a conserved mechanism involving Rtf1 in cardiogenesis from fish to mouse.

295. The novel zebrafish mutant *sea squirt* is a suppressor of heart size in retinoic acid deficient embryos. ***Amrita Mandal***[1,2], *Joshua Waxman*[2]. 1) Molecular and Developmental Biology Graduate Program, College of Medicine, University of Cincinnati,; 2) Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, USA.
Proper heart size is necessary for the normal function of heart during embryonic and postnatal life. Retinoic acid (RA) signaling plays critical roles in different phases of normal heart development, with RA signaling being one of the few signaling pathways necessary to restrict cardiac specification. However knowledge about molecular networks that cross talk with RA in determining appropriate embryonic heart size is not yet well understood. We conducted a F2 modifier screen in zebrafish to identify mutants that suppress the enlarged hearts of RA signaling deficient *neckless (nls)* embryos. We found a novel *nls* suppressor mutant called *sea squirt*. Importantly in the *nls;sqi* double mutants, heart size and morphology are largely restored to normal. Alone *sqi* mutants have mildly dysmorphic hearts. While our preliminary data indicate that there is increased apoptosis in *sqi* mutants, we did not observe cell death in heart, suggesting that cell death might not be the primary mechanism behind heart size suppression. Next, to decipher the molecular nature of the lesion in *sqi*, we performed traditional positional cloning in combination with recently developed RNA-seq based mapping (RNA mapper). This approach allowed us to narrow the mutation to a region spanning 3mb on chromosome 1. To identify the gene affected in *sqi* we are in the process of examining candidate genes within the region using PCR (qRT-PCR), *in situ* hybridization (ISH) and rescue studies with mRNA overexpression. Deciphering the underlying molecular and genetic networks by which the *sqi* mutation suppresses the enlarged heart of RA deficient embryos will shed light on the regulation of heart size in vertebrate embryos and may lead to novel understating of CHDs in humans.

296. A Zebrafish Model of Cardiorenal Syndrome. ***Steve Mangos***[1], *Nicholas Tardi*[1], *Myles Wolf*[2], *Jochen Reiser*[1]. 1) Internal Medicine, Rush University Medical Center, Chicago, IL; 2) Center for Translational Metabolism and Health, NorthWestern University Feinberg School of Medicine, Chicago, IL.
Cardiorenal syndrome dates back to at least 100 years ago. In recent years, an exciting physiological link has been made between the renal and endocrine systems regulating phosphate homeostasis and Left Ventricular Hypertrophy (LVH). Phosphate regulation is maintained in most animals under the control of the FGF23-Klotho-vitD axis. In humans, high levels of FGF23 has been associated with chronic kidney disease and these patients have increased mortality due to cardiovascular disease. Recent work in cell culture and mammalian models has shown that the cardiovascular disease predominantly manifests as LVH and is directly or indirectly caused by elevated levels of FGF23. Our goal is to utilize the zebrafish to further understand the physiological and molecular mechanisms that link cardiac hypertrophy and Fgf23 signaling. We have made progress in determining how zebrafish regulate phosphate levels by context-specific characterization of several phosphate transporters. We have previously characterized zebrafish *fgf23* and show that it is expressed as a paracrine factor in the Corpuscles of Stannius. We now show that, similar to the mammalian system, elevated levels of *fgf23* has effects on the heart in zebrafish larvae. We have developed various transgenic lines to elevate Fgf23 signaling in a global as well as a tissue specific fashion. Our current work aims at identifying the specific Fgf receptors as well as the downstream pathways initiated by Fgf23 signaling in the heart. We will use our findings as the basis to develop a screening approach to uncover novel therapies to treat or prevent certain forms of cardiorenal syndrome.

297. Localization and function of small heat shock protein Hspb7 in cardiovascular and laterality development. *John Sanderson*[1], *Kaijie Zheng*[1], *Mark Springel*[1,2], *Jonathan Wosen*[1], ***Martha Marvin***[2]. 1) Biology Department, Williams College, Williamstown, MA; 2) Neuroscience Program, Williams College, Williamstown, MA.
Small heat shock protein *hspb7* is essential for both the establishment of left-right asymmetry and for myocardial function. Kupffer's vesicle (KV) is a ciliated organ that is necessary for the propagation of left-right asymmetry. Hspb7 protein is present in KV as small punctae associated with the developing cilia, but begins to be excluded from KV by 12 hpf. Hspb7 and KV become spatially separated over time, and Hspb7 becomes associated with cytoplasmic aggregates at some distance from KV. The association of the developing KV cilia with Hspb7 and other KV specific proteins will be explored. *hspb7* has been shown to affect myocardial function. We have demonstrated that *hspb7* knockdown increases the size of heart valves. Given that *hspb7* is expressed both in the yolk syncytial layer and in the myocardium, the effect on valves could be a direct effect on the heart, or an indirect effect via extra-embryonic expression in the YSL. Our data suggests that the requirement for *hspb7* in valve development depends on its early, YSL expression rather than expression in the developing heart. This suggests that the myocardial defects, which are also dependent on expression in the YSL, contribute to the valve overgrowth in *hspb7* morphants.

298. Hace1 influences oncogenesis and vertebrate cardiac development via ROS-dependent mechanisms. ***Babak Razaghi***[1], *Shelby L. Steele*[2], *Lindsay McDonald*[3], *William Lin*[1], *Mads Daugaard*[5,6], *Ian C. Scott*[7], *Poul H.B. Sorensen*[5,8], *Jason N. Berman*[1,2,4]. 1) Departments of Microbiology & Immunology; 2) Pediatrics; 3) Emergency Medicine; 4) Pathology, Dalhousie University/IWK Health Centre, Halifax, NS, Canada; 5) Department of Molecular Oncology, British Columbia Cancer Research Centre, Vancouver, BC; 6) Vancouver Prostate Centre, Vancouver BC, Canada; 7) Hospital for Sick Children and University of Toronto, Toronto, ON, Canada; 8) Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada.
HACE1 is a tumor suppressor gene located on chromosome 6q21 that is expressed in various tissues including heart, brain and kidney. HACE1 expression is downregulated in sporadic Wilms' tumor and other cancers. However, the mechanism underlying tumorigenesis and the role of HACE1 in normal vertebrate development have not been well-elucidated. We have recently shown that HACE1 regulates production of reactive oxygen species (ROS) and DNA damage through its interaction with its only known ubiquitination target, the small Rho family GTPase, Rac1 (Daugaard et al, *Nat. Commun* 2013). Rac1 is a modulator of cellular ROS generation that localizes to the NADPH oxidase (NOX) holoenzyme. More recently, we have found that loss of hace1 in zebrafish via morpholino knockdown results in an increased incidence of cardiac looping defects, where the heart is either tubular or the positions of the atrium and ventricle are reversed (inverted). Whole mount *in situ* hybridization (WISH) of heart-specific markers shows distinct abnormalities in ventricular morphology and atrioventricular valve formation in the hearts of these morphants, as well as increased expression of rac1. Interestingly, this phenotype also appears to be directly related to Nox enzyme-dependent ROS production as both genetic inhibition by nox morpholinos or treatment with ROS scavenging agents restores normal cardiac structure (Razaghi et al, submitted). Our data reveal a conserved molecular mechanism that controls the activity of Rac1-dependent NOX complexes both in cancer and normal cardiac development, and thus constitutes the first known example of a tumor suppressor protein that regulates a developmental process in vertebrates.

299. Exposure to environmental estrogens octylphenol causes deficiency of cardiovascular development in zebrafish. *Rong-Guang Wang, Chia-Hung Yen,* ***Shao-Yang Hu***. Department of Biological Science and Technology, National Pingtung University of Science and Technology. Pingtung, Taiwan.
The environmental estrogen, octylphenol (OP), which belong to alkylphenol ethoxylates (APEOs), is existed mainly as intermediates in the manufacturing industry. Animals expose to OP frequently result in reproductive defects due to the structure and activity similar to estrogen. Recent clinical reports showed that OP was detected in umbilical cord blood of newborn suggesting expectant mothers expose to OP leading to the contaminations of fetus through blood delivery. Although several reports have shown that animals exposure to OP cause the feminization of male, sterility and deficiency of gonad development, less know about the effects of OP on embryonic development of fetus. In the present study, the influences of OP on fetus were investigated by zebrafish embryos exposure to OP. Results showed that the survival rate was 71.2±2.8%; (n=180) with 1 uM of OP treatment for 3 days, and 60.4±1.3%; of survival embryos show the cardiovascular defects including cardio edema and irregular shape of atrium and ventricle, slower heart rate and blood flow rate, pooled blood in caudal vein and abnormal vessel development. The transcript of nkx2.5, GATA family, tbx-5 and dHand genes, which are cardiovascular related transcription factors, are decreased after OP treatment. The OP treatment casues deficiency in nkx2.7 expression and leads to failed looping and defective chamber in heart was showed by whole mount in situ hybridation. These results elucidated that environmental estrogen OP would cause the deficiencies in cardiovascular development and functions. We expect the results can be as references relevant to the clinic reports of fetus exposure to environmental estrogens.

300. Molecular phenotyping of small molecule treated zebrafish embryos. ***Zsofia Pusztai****, Christopher M. Dooley, John Collins, Neha Wali, Ross N.W. Kettleborough, Elisabeth M Busch-Nentwich, Derek L. Stemple.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
Our study aims to examine the developmental and toxicological effects of small molecules on zebrafish embryos on a morphological and transcriptional level. Transcriptional changes induced by these compounds are analysed to uncover genetic interactions and their accompanying networks. This is accomplished using our transcriptomic analysis pipeline called DeTCT (Differential expression transcript counting technique), which is a high-throughput sequencing based approach for generating quantitative transcriptome profiles from total RNA samples. Wild type zebrafish embryos were treated with different concentrations of small molecules during the first five days of development. Treated and control embryos were collected at several developmental stages and submitted for transcript counting experiments. Identification of the morphological differences was obtained using standard light microscopy. Morphological changes and staging are annotated according to zebrafish stage and anatomy ontologies at the time of collection. Small molecule screens have traditionally been used as an approach to reveal specific targets in drug discovery. We aim to discover novel genetic networks that play a role in embryonic development and disease therapy. Importantly, the study of the effects, both on and off target, is not limited to certain cell or organ types but effects of the different compounds can be screened on the whole organism in a high throughput manner.

301. Identification of chemical vascular disruptors during development using an integrative predictive toxicity model and zebrafish and in vitro functional angiogenesis assays. ***Tamara Tal***[1], *Nicole Kleinstreuer*[2], *Tarja Toimela*[3], *Riina Sarkanen*[3], *Tuula Heinonen*[3], *Thomas Knudsen*[4], *Stephanie Padilla*[1]. 1) Integrated Systems Toxicology Division, U.S. EPA, RTP, NC; 2) ILS, inc./NICETUM, RTP, NC; 3) FICAM, University of Tampere, Tampere, Finland; 4) National Center for Computational Toxicology, U.S. EPA, RTP, NC.
Chemically induced vascular toxicity during embryonic development can result in a wide range of adverse prenatal outcomes. Previously, we constructed an embryonic vascular disruption adverse outcome pathway (AOP) based on molecular initiating events corresponding to genetic models with phenotypic evidence of abnormal embryonic vascular development in the Mouse Genome Informatics Database. Here we used ToxCast high throughput screening data for 25 assays mapping to targets in the Vascular Disruption AOP to prioritize 1060 chemicals for their potential to disrupt vascular development. A subset of 37 predicted vascular disrupting chemicals (pVDCs) or non-pVDCs, including pesticides, flame retardants, and endocrine active compounds, were selected for targeted testing in zebrafish (*D. rerio*).

To test computational predictions, TG(flk1:GFP) zebrafish embryos were used to visualize and quantify blood vessel formation during development. Manual and automated methods of vessel quantification were developed, and the assay was evaluated with anti-angiogenic reference compounds PTK787 and AG1478, small molecule inhibitors of VEGFR2 and EGFR, respectively. The zebrafish assay was then used to test the effects of 37 chemicals in combination with a functional angiogenesis assay comprised of a human endothelial cell and fibroblast co-culture system. Chemical rankings were well correlated among the predictive signature and zebrafish and *in vitro* tubulogenesis assays. Taken together, the zebrafish assay meets a critical need for an *in vivo* platform that can assess predictions generated by computational models of developmental vascular toxicity. *This abstract does not necessarily reflect EPA policy.*.

302. A New Role of YAP in Primary Cilia Development in Retina. ***Na Luo***[1], *Clark Wells*[2], *Ryan Anderson*[3], *Yang Sun*[1]. 1) Department of Ophthalmology, Indiana University, Indianapolis, IN; 2) Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN; 3) Department of Pediatrics, Indiana University, Indianapolis, IN.
Hippo pathway consists of a large network of proteins that control the growth of different tissue during development and regeneration, as well as in pathological state such as cancer, through restraining cell proliferation and promoting apoptosis. Yes-associated protein 65 (YAP), is one of the major downstream effectors of the hippo pathway, which expressed in a wide range of tissues, except peripheral blood leukocytes. Hippo signaling has been recently linked to ciliogenesis through the interplay with other established signaling cascades; however, the role of YAP to ciliogenesis is not known. Through in situ hybridization assay, YAP was detected to localize in the otic vesicle and ocular tissues during zebrafish early embryogenesis. In the retina of rat posterior eye, strong immunoreactivity of YAP was found in the outer segments of retinal photoreceptors that are modified ciliary organelles. YAP zebrafish morphants generated by transient knockdown with specific anti-sense morpholino oligonucleotides presented microphthalmia, smaller brain, kinked tail, pronephros cysts, edema, and heart left-right asymmetry. Compared to control morphants of 5dpf zebrafish larvae, YAP morphants developed thinner outer segments cells in retina photoreceptors. Kupffer's vesicle cilia in YAP morphants were found less and shorter than control morphants. In serum starved hTERT-RPE cells, endogenous pYAP was observed to localize in primary cilia as well as cytoplasmic. Compared to control, cilia lengths were much shorter in YAP stable knockdown hTERT-RPE cells generated by lentiviral transduction. These results show that YAP may localize within the cilia and associate with primary cilia development in retina, and provides a role of Hippo signaling in primary cilia development.

303. The Transcription Factor *etv5a* is Essential for Multiciliated Cell Development in the Zebrafish Pronephros. ***Amanda Marra***, *Rebecca Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
The zebrafish pronephros, or embryonic kidney, is comprised of two main types of epithelial cells: transportive and multi-ciliated cells (MCCs). Transportive cells are characterized by their expression of ion transporters, while MCCs are dispersed in a "salt-and-pepper" fashion throughout the proximal convoluted tubule (PCT), proximal straight tubule (PST) and distal early (DE) segments, with the densest numbers present in the PST. The fate choice between transportive and MCC cell type is regulated by Notch signaling, in which Notch activity promotes transportive cell identity by restricting MCC fate choice. Recent research has demonstrated that the transcription factor *mecom* acts to restrict MCC identity by promoting Notch activity, and that retinoic acid (RA) signaling is required upstream to regulate *mecom* expression. Despite this knowledge, there is little known about MCC development. Previous research has shown that *etv5a* and its ETS family members *etv5b* and *etv4* are required for ciliogenesis of mono-ciliated cells in Kuppfer's Vesicle (KV) during zebrafish development. Here, we mapped *etv5a* expression to renal progenitors that occupy domains where MCCs later emerge. Thus, we hypothesized that *etv5a* is required for ciliogenesis of MCCs in the zebrafish pronephros. Morpholino knockdown of *etv5a* and whole mount in situ hybridization (WISH) analysis have shown that the loss of *etv5a* is accompanied by a decreased expression of the MCC markers *odf3b* and *centrin*, suggesting that *etv5a* is required for MCC formation. Furthermore, *etv5a* morphants develop edema after 36hpf, suggesting kidney failure. Ongoing studies will examine the epistatic relationship of *etv5a* to RA, *mecom* and Notch to further establish the mechanism of how these factors interact to direct MCC development in the pronephros.

304. Investigating the role for Tmem14a in planar cell polarity, development and disease. ***M. Mendoza***[1,2], *B. Ciruna*[1,2]. 1) Hospital for Sick Children, Toronto, Ontario, Canada; 2) University of Toronto, Department of Molecular Genetics, Toronto, Ontario, Canada.
The planar cell polarity (PCP) pathway controls numerous biological processes, each of which involves the synchronous movement and organization of cells within a single plane of tissue. Regulation of the PCP pathway is largely attributed to a core set of signaling molecules, such as Vangl2. Although much work has been done to characterize PCP, there remain many unknowns about the pathway, such as the factors contributing to the initial establishment and regulation of component asymmetric distribution. Recently, we identified the transmembrane protein Tmem14a as a novel interactor of Vangl2. Initial functional analysis suggests that Tmem14a localizes to a polarized compartment of the trans-Golgi, and that it participates in the posterior positioning of basal bodies within the zebrafish neural tube. Additionally, tmem14a mutants possess Golgi fragmentations within enveloping layer cells, suggesting that Tmem14a may play a role in Golgi structuring. Based on the localization of Tmem14a to the Golgi and evidence of an interaction with Vangl2, we hypothesize that Tmem14a may also be a factor in Vangl2 trafficking. Here, we present our ongoing studies (1) investigating the role of Tmem14a in the trafficking of PCP signaling components and (2) determining how Tmem14a function contributes to Golgi complex organization.

305. Mechanism for microtubule-dependent dorsal determination in zebrafish. ***Hiromu Hino***[1], *Ryoko Seki*[1], *Takashi Shimizu*[1], *Francisco Pelegri*[2], *Masahiko Hibi*[1]. 1) Nagoya University, Nagoya, Aichi, Japan; 2) University of Wisconsin, Madison, USA.
In zebrafish embryos, dorsal determinants are believed to be initially localized to the vegetal pole and then transported to the future dorsal side of the embryo along a microtubule array. The dorsal determinants activate the canonical Wnt pathway and promote the expression of genes that are required for the formation of dorsal structures. We previously demonstrated that Syntabulin, a microtubule-associated protein, is required for the dorsal determination. Using transgenic lines expressing EGFP-Tubulin and EB1-GFP, we found a parallel

microtubule array formed at the cortical surface of the vegetal pole at around 20 min post fertilization (mpf) and then disappeared 10 min after. Imaging of EB1-GFP comets showed that the plus end of the microtubules was directed to the future dorsal side. FRAP analysis revealed that the vegetal-pole microtubules themselves moved along with vesicular structures to the future dorsal side, suggesting that the dorsalward movement of the vegetal microtubules is involved in the transport of the dorsal determinants. We found that Syntabulin-GFP, expressed by RNA injection into an oocyte, surrounded the vesicles and moved to the future dorsal side during the period of the microtubule formation. Biochemical analysis revealed that Syntabulin could interact with a glutamate receptor interacting protein 2a (Grip2a). Syntabulin and Grip2a showed distinct localization when they are expressed alone in blastomeres. However, Syntabulin was co-localized with Grip2a on microtubules when they were co-expressed. Immunostaining showed that endogenous Syntabulin and Grip2a were co-localized on microtubules and on the surface of the vesicles at the vegetal pole. These data suggest that (1) the moving polarized microtubules provide Syntabulin with a positional cue for the directional transport; (2) Syntabulin interacts with Grip2a to recruit the vesicles containing the dorsal determinants onto the vegetal microtubules; (3) the Syntabulin-Grip2a complex transports the dorsal determinants to the dorsal side.

306. Zebrafish myca and mycb are distinguishable genes for embryonic development and growth. ***Wenshuang Jia****, Xiaoxiao Wang, Shasha Cao, Zhangji Dong, Xiaohua Dong, Qingshun Zhao.* Model Animal Research Center, MOE Key Laboratory of Model Animal for Disease Study, Nanjing University, Nanjing 210061, China.
Myc, a well-known myelocytomatosis oncogene, has been proved to play important roles in a variety of cell processes including cell-cycle progression, cell proliferation, cell differentiation, survival, adhesion, and cell-size determination. Homozygous *Myc* mutant mice die on or before 10.5 days of gestation. In human patients with tumor, *MYC* is found to be down regulated in many malignant tissues. However, little is known about its roles in zebrafish early development. In order to understand the roles of *myc* in zebrafish embryogenesis, we knocked out zebrafish *myca* and *mycb*, which are orthologues of human *MYC*, by using ZFN (zinc finger nuclease) technology or TALEN (transcription activator-like effector nuclease) technology, respectively. Phenotypic analyses revealed that the *myca* or *mycb* null embryos, and *myca* and *mycb* double null embryos developed and grew normally to sex mature. The results suggest that *myca* and *mycb* are distinguishable genes for zebrafish to develop and grow.

307. Wnt pathway regulates hindbrain size specification and patterning in zebrafish. ***Spencer Keil****, Isaac Skromne.* Dept. of Biology, University of Miami, Coral Gables, FL.
The partitioning of the caudal neuroectoderm into an anterior hindbrain and a posterior spinal cord culminates vertebrate central nervous system regionalization. Here we investigate the function of Wnt signaling factors in regulating the partition of hindbrain and spinal cord territories. To determine if Wnt is necessary for regulating the size of the hindbrain territory, we blocked the Wnt canonical pathway by inducing global overexpression of the inhibitor Dkk1. Temporal control was achieved by using a transgenic line containing a heat shock inducible Dkk1 gene. When inhibition of the Wnt pathway was initiated before gastrulation, the expression domain of the spinal cord specification gene Cdx4 relative to the head/trunk transition at somite 2/3 remained unchanged, suggesting that the alignment of neural and paraxial mesoderm tissues at the head/trunk transition was not affected. However, relative to head landmarks, this manipulation shifted the position of the head/trunk transition anteriorly, indicating a reduction or a truncation in caudal hindbrain territory. To determine whether the shift was due to reduction or truncation of more posterior rhombomeres, we analyzed the expression of hindbrain patterning genes. Using various markers of posterior rhombomere fates, we found that the Wnt-deficient embryos have a fully patterned hindbrain, but that rhombomeres 5-7 were noticeably reduced. Together, our data show that Wnt signaling is required during gastrulation for hindbrain size specification and/or growth. Critically, Wnt inhibition at mid-gastrulation stages showed no such size reduction, suggesting that Wnt function in hindbrain territory specification is restricted to the first half of gastrulation. This result is significant, as work from our lab has shown that another signaling factor involved in hindbrain territory specification, Retinoic Acid, is required throughout gastrulation for proper hindbrain territory size. Understanding the mechanism of hindbrain and spinal cord territory specification by Wnt and its interactions with other signaling factors would be important for elucidating how the neuroectoderm is partitioned, patterned and aligned to mesoderm tissue at the head-trunk transition.

308. The Role of Bucky Ball in Germ Cell Specification. ***Pritesh Krishnakumar****, Roland Dosch.* Developmental Biochemistry(Inst.Entwicklungsbiochemie), Georg-August-Universitaet, Goettingen, Niedersachsen, Germany.
Reproduction and germ cell formation are important processes for the continuation of a species. In zebrafish, germ cells are specified by germ plasm during the development of the embryo. Our lab discovered the bucky ball (buc) gene in Zebrafish, which is the first known vertebrate gene assembling germ plasm. buc also mimics germ plasm activity i.e. overexpression reprograms somatic cells into germ cells. Since the Buc protein sequence shows no homology to other functional domains, its biochemical activity is unknown. Our main goal is to characterize the molecular mechanism of germ cell specification by Buc. More specifically, we will identify interactions of Buc and its domains responsible for the formation of germ cells. Two zebrafish mutant alleles, p106 and p43 disrupt germ plasm formation in the oocyte. Sequencing both alleles identified premature STOP-codons deleting the C-terminal 37 and 277 amino acids of Buc. However, the process disrupted by these mutations is currently unknown. To functionally dissect the Buc protein, we started to investigate the activity of these C-terminal ends. When we co-injected these mutant Buc proteins with GFPnos 3'UTR into the embryo, no ectopic germ cells are induced. This result shows that the C-terminus is essential for inducing ectopic germ cells. Hence, the germ cell inducing activity of Buc might be present in the C-terminal end. If this end is also sufficient to induce ectopic germ cells, we will use this domain to search for potential interactors using co-immunoprecipitation methods. Interestingly, injecting the Drosophila germ plasm organizer Oskar in zebrafish also forms additional germ cells suggesting that the molecular program of germ cell formation is conserved between invertebrates and vertebrates. In summary the results of this project will provide a biochemical mechanism for the evolutionary conserved process of germ cell formation, which is initiated by Buc.

309. Lzap Controls Dorsal-Ventral Patterning via The Regulation of NF-kB on BMP-Chordin Axis. ***K.Y. Lin***[1], *C.T. Chen*[3], *A.P. Anugra Yekti*[2], *W.D. Wang*[2], *H.J. Hsu*[1]. 1) Institute of Cellular and Organismic Biology, Academia Sinica, Taipei,Taiwan; 2) Department of BioAgricultural Science, NCYU, Chiayi, Taiwan; 3) Institute of Bioinformatics and Structural Biology, NTHU, Hsinchu, Taiwan.
Drosal-ventral patterning in vertebrates is relied on the ventral to dorsal BMP signaling gradient, which is established by the dorsal BMP antagonists (e.g. Chordin). However, very little known about how the expression of those antagonists is controlled. Here, we report that Lzap, known as a tumor suppressor via the suppression of NF-kB activity, limits dorsal expression of chordin to generate BMP signaling gradient probably through the same mechanism. Elimination of lzap expression in embryos using antisense morpholino oligos increases the expression of chordin without affecting bmp2b expression at the stage before BMP signaling gradient is established. In addition to chordin, expressions of several downstream targets of Wnt/b-catenin and NF-kB signaling, as well as fgfs are also increased in lzap- knocked down embryos. Further, b-catenin nuclear localization and FgF signaling are also promoted in these embryos. Consequently, at later development stages these embryos with decreased expressions of bmps are dorsalized. Interestingly, overexpression of bmp2b mRNA or suppressed NF-kB signaling rescues the dorsalization phenotype of lzap-knocked down embryos. We are currently examining the requirement of Wnt/b-catenin and FGF signaling on chrodrin transcription mediated by Lzap, and the involvement of NF-kB signaling in this process. Nevertheless, our results here have uncovered a new upstream regulator for the BMP/Chordin axis to pattern the body plan.

310. Dissecting the differentiation process of the pre-placodal ectoderm in zebrafish. *Ying Wu, Feng Zhao, Di Yao, Jialiang Wang, Jue Zhao, Zuoyan Zhu,* ***Dong Liu***. The Education Ministry Key Laboratory of Cell Proliferation and Differentiation, School of Life Science, Peking University, Beijing, China.
The pre-placodal region (PPR) is specialized ectoderm at the border of neural and non-neural ectoderm (NNE). It's known that coordinated Bmp, Fgf and Wnt signals drive the PPR development, yet the underlying mechanism remains to be explored. We have identified key components that implement PPR differentiation. The mesoderm/marginal Wnts at the early gastrula (5hpf) trigger the process by allowing adjacent NNE border cells to start adopting caudal PPR fate; otherwise the gain of caudal identity is hindered, due to the persistent presence of *gata3* mRNA. The caudal PPR fate dominates when *foxi1* expression is enhanced at the late gastrula and depleting Foxi1 after 6hpf particularly reduces the otic-epibranchial placodal domain. When Gata3 level is manipulated at the fertilized egg stage or around 6hpf, the lens is always affected. In establishing the PPR polarity, both Gata3 and Foxi1 inhibit Bmp signaling, while Foxi1 inhibits but Gata3 enhances Fgf sensitivity of the PPR cells. Our study reveals 1) the NNE border cells at the shield stage already step into a differentiating state, and 2) a network of genetically-linked factors that directs the process of PPR differentiation.

311. KLF4 is downstream of IRF6 in the periderm gene regulatory network and is a candidate human clefting locus. ***H. Liu***[1], *T. Smith*[1], *Z. Jia*[2], *J. Murray*[2], *R. Cornell*[1]. 1) Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.
Mutations in interferon regulatory factor 6 (IRF6) account for ~70% of cases of Van der Woude syndrome and some of the non-syndromic orofacial clefting. In mice Irf6 is necessary for differentiation of the most superficial layer of oral epithelium, the oral periderm. We found IRF6 is required for the differentiation of the zebrafish embryonic periderm and have used this tissue as a model for human oral periderm. We have reported that Krüpple-like factor 4 (KLF4) is expressed in the periderm of zebrafish, and its expression is inhibited by the dominant-negative variant of IRF6. Based on these preliminary data, we hypothesized that as a downstream of IRF6, KLF4 might play a role in the differentiation of periderm. Over expression of klf4 rescues periderm marker expression in embryos injected with dnIrf6, supporting this model. Next we hypothesized that mutations in KLF4 may predispose people to orofacial clefting. To test this possibility we sequenced the exons of KLF4 among cases and controls from Philippines (604 patients with Non syndromic CLP, 182 with NSCL and 343 Control), Iowa (180 with NSCLP and 69 with NSCL), and Ethiopian (25 with NSCLP, 47 with NSCL and 5 with NSCP). The sequencing results showed there were 11 missense mutations from these NSCL/P samples, 5 of which were not presented in the control. To screen whether these patient-derived variants are pathogenic, we are utilizing the zebrafish periderm differentiation model. Forced expression of wild-type human KLF4 induces ectopic expression of Krt4 in the deep layer cells of the embryo during epiboly stages. We are currently testing patient-derived variants to determine if their function differs from wild-type. Our data illustrate that KLF4 is downstream of Irf6 in the gene regulatory network governing zebrafish periderm differentiation suggesting it is a candidate risk locus for oral clefting. The IRF6-related periderm gene regulatory network in zebrafish is a powerful tool in predicting the risk loci in the superficial-epithelium related diseases, including orofacial clefting.

312. Eaf1 and Eaf2 Are Required for TGF-b Genes Response by Cooperating With Smads and p53. ***J.X. Liu***[1], *T. Zhang*[1], *Y. Zhang*[2], *W. Xiao*[2], *J.F. Gui*[2]. 1) Huazhong Agricultural University , Wuhan, Hubei, China; 2) Institute of Hydrobiology, CAS, Wuhan, Hubei, China.
EAF family genes act in multiple cellular responses including tumor suppression and embryonic development. In zebrafish, Eaf1 and Eaf2 modulate convergence and extension movements by maintaining the Wnt non-canonical ligands wnt5 and wnt11, and modulate embryonic DV and AP patterning by negatively regulating canonical Wnt/b-catenin signaling. Here, we report that the key cellular responses to TGF-b signals require EAF gene family members. In zebrafish embryos of gain-of-function of eaf1 or eaf2, multiple TGF-b target genes, which are mesoendederm markers, display reduced expression. Moreover, in embryos knockdown signal eaf1 or eaf2 or both, those markers displays enhanced expression, and double knockout of eaf1 and eaf2 can rescue expression of gata5 and cas in MZoep mutant fish. Furthermore, we find that in mammalian cell lines, knockdown Eaf1 or Eaf2 expression by siRNA resulted in increased expression of TGF-b targets, on the contrary, expression of the targets were suppressed in cells with stable Eaf1 or Eaf2 over-expression. Eaf1 and Eaf2 and its mammalian homologs interact with the P53 and smad proteins, thereby inhibiting TGF-b target gene induction and mesoendoderm differentiation, and Eaf may respectively cooperate with smad and p53 protein complexes converge on separate cis binding elements on gata5 promoter and synergistically suppress TGF-b induced transcription. The results unveil a previously unrecognized link between Eaf genes and TGF-b pathway in vertebrates.

313. Canonical Wnt, FGF, and BMP signaling interact to pattern axial stem cell derived mesoderm. *Brian Kinney, Richard H. Row, **Benjamin L. Martin***. Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY.
Vertebrate embryos form their body through a process called posterior growth, where the head forms first and the rest of the body develops progressively towards the posterior end. After gastrulation, a population of axial stem cells in a structure at the posterior-most end of the embryo called the tailbud, fuels the process of posterior growth by contributing cells to newly forming tissues. Axial stem cells make a basic germ layer decision between neural ectoderm and mesoderm, but it is unclear how and to what extent the newly formed mesoderm is patterned. We used heat-shock inducible transgenes to temporally inhibit or activate the canonical Wnt, FGF, and BMP pathways during tailbud stages and examined mesodermal cell fate. We found that Wnt and FGF act at different steps to promote dorsal (paraxial) mesoderm formation and inhibit ventral (vascular endothelial) mesoderm, while BMP has a reciprocal effect, promoting ventral and inhibiting dorsal mesoderm formation. Additionally, we find that within the paraxial mesoderm, somite fate (which gives rise primarily to skeletal muscle and bone) is not determined until immediately prior to physical somite formation. Our results indicate that axial stem cells generate basal mesoderm that remains highly plastic, and is patterned through a complex interaction between Wnt, FGF, and BMP signaling.

314. Relevance of state of ploidy during early development of vertebrate embryos. *Triveni Menon, **Sreelaja Nair***. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India.
In animals, deviations from diploidy are incompatible with embryonic viability. The number of chromosomes in a cell is its ploidy and alterations in cellular ploidy post-embryogenesis often indicate disease states. Despite this, mosaic non-diploidy is contextually tolerated and even considered beneficial for an organism (hepatocytes and megakaryocytes are naturally polyploid and cardiomyocytes under stress have the potential to become polyploid). In zebrafish, unlike mammals, deviations from diploidy at fertilization do not immediately elicit lethality. Non-diploid zebrafish embryos appear to undergo normal early cell divisions and embryogenesis. However, despite this apparently normal early development, non-diploid zebrafish embryos do not survive beyond 7-8 days post fertilization. The cause of lethality remains unknown. We hypothesize that non-diploidy associated lethality, which manifests late in development has its genesis in cell biological and molecular errors that occur within the first few hours after fertilization. Our experiments reveal alterations in the cell biological machinery in non-diploid zebrafish embryos as early as the one-cell stage, the earliest developmental time point at which any defects have been observed in non-diploid embryos. Such early defects are somewhat surprising since non-diploid embryos do execute the early developmental program, albeit a faulty one. We have uncovered global patterning defects particularly in axis specification, germ layer formation and gastrulation, all of which together with temporal deviations in zygotic genome activation potentially contributes to lethality. We currently use non-diploid zebrafish embryos to understand the consequences of mosaic ploidy in live embryos by engineering zebrafish embryos of mosaic ploidy at key developmental stages. Our study aims to provide a comprehensive understanding of non-diploidy associated lethality in a normally diploid animal species, and allows a mechanistic approach towards uncovering the cellular behavior of a population of cells of mixed ploidy, a hallmark of several disease states.

315. Zebrafish Melanophore Integrity Mutants and Their Implications for a Melanosome-Mediated Drug Sensitivity Model. ***Kersten A Peterson***, *Lauren Clancey, Cynthia Cooper*. Washington State University, Vancouver, WA.
Zebrafish melanophores (black pigment cells) share a high degree of homology with human melanocytes and have been extensively used as a model for studying human melanocyte development and function. Here we present preliminary data characterizing two loss of function genetic mutations that impact Zebrafish melanophore function and integrity as well as affect overall embryo health and survival. The mutations in these Zebrafish lines affect the structures and function of vacuolar protein sorting (Vps) complex members: Vps11 and Vps16. These proteins function as part of the Class C Vps protein complex and are a critical trafficking component of Endosome to Lysosome and Endosome to Melanosome transport. This complex forms the core of trafficking complexes HOPS and CORVET and works closely with SNARE during docking and fusion. Previous work characterizing Vps11 in Zebrafish found that melanophore death in mutant embryos is caspase independent and that Vps11 functions by inhibiting cathepsin activity, allowing the formation of normal melanophore morphology. Preliminary observations show the Vps16 mutant phenotype to be similar to that of Vps11 apart from increased severity of phenotype in melanophore survival and iridophore number. This was expected as Vps16 contains an active site in the complex where Vps11 does not. Current work is further characterizing the Vps11 mutation with a drug screen, in which we hope to find a rescuing agent for the phenotype and to compare drug sensitivities between wildtype embryos and mutant embryos. Members of the Class C Vps protein complex have been shown to play a role in melanosome mediated chemotherapy drug resistance. In the future we hope to use these two Zebrafish lines as models for the study of pigment cell sensitivity to melanoma chemotherapy.

316. Isl1 is a key factor of late enteroendocrine cell differentiation. ***J. Pirson***, *L. Flasse, D. Stern, B. Peers, M. Voz.* GIGA-Research-Unit of Zebrafish Development and Disease Models, University of Liège, Avenue de l'Hopital, 1 (B34) 4000 Liège Belgium.
Recently, it has been shown that foxo1 somatic ablation in the gut give rise to insulin-producing, glucose responding cell. This brings the challenging idea that gut stem cell could be used as a source for insulin cells replacement. This strategy will necessitate a profound knowledge of the differentiation pathway of the enteroendocrine cells. In this study, we focused on the islet-1 (isl1) transcription factor which is essential for the differentiation of all murine endocrine pancreatic cells but its function is unknown in the intestinal tract, only isl1 expression has been showed in the gastrointestinal epithelium in developing and adult mice. Like in the pancreas, isl1 is not expressed in the precursor cells but only in all majority of mature enteroendocrine cells. Loss-of-function analyses using the isl1 mutant sa0029 revealed that isl1 blocks the tardive differentiation of the cells which remain in the progenitors state and do not express the enteroendocrine hormones. This study shows that isl1 acts at late stage in the differentiation process of the enteroendocrine cells in the zebrafish intestine, playing a similar role as in mouse and zebrafish pancreas. These results show a strong conservation intra-tissue and intra-species in the differentiation process of endocrine pancreatic cells and enteroendocrine cells.

317. Modeling renal coloboma syndrome: humanizing *pax2a* alleles in zebrafish. ***Pamela R. Pretorius***[1], *Stephanie L. Lerach*[1], *Staci L. Solin*[2], *Maura McGrail*[2], *Lisa A. Schimmenti*[1]. 1) Department of Pediatrics and Department of Genetics, Cell biology and Development, University of Minnesota, Minneapolis, MN; 2) Department of Genetics, Development and Cell Biology, Iowa State.
A pleiotropic autosomal dominant human disease, renal coloboma syndrome results from mutations in the transcription factor *PAX2* and is characterized by renal and eye malformations. Malformations include hypodysplastic kidneys, optic nerve dysplasia and occasionally retinal coloboma. Why mutations in *PAX2*, a developmental regulator for kidney, midbrain/hindbrain, spinal column, otic vesicle, eye and spinal neurons, predominantly impacts kidney and eye development is unclear. One factor could be that nearly all human mutations are located in the paired domain, which through DNA interactions can act as either a transcriptional repressor or activator. These observations led us to hypothesize that mutations in the paired domain impact *PAX2* function during early development. To generate *pax2a* paired domain mutations, TALEN constructs targeting exon 2 of *pax2a* were microinjected into one-cell embryos. In the F1 generation, five novel *pax2a* mutant alleles were recovered and included both frameshifts and in frame deletions. Two lines, $pax2a^{+1/+1}$ and $pax2a^{D9/D9}$ were characterized. $pax2a^{+1/+1}$ and $pax2a^{D9/D9}$ animals were identified at 1dpf due to lack of isthmus formation and confirmed through genotyping. By 3dpf, $pax2a^{+1/+1}$ embryos had small eyes, cardiac edema, and kidney edema. Moreover, less than 10% of the $pax2a^{+1/+1}$ animals survived to 7dpf, consistent with previously published $pax2a^{tu29/tu29}$ allele data. In contrast, 3dpf $pax2a^{D9/D9}$ embryos were indistinguishable from wild-type and demonstrated over 70% survival to 7dpf. Histological analysis of $pax2a^{+1/+1}$ animals at 3dpf revealed reduced eye size, hypocellularity and optic nerve dysplasia. Retina lamination was preserved; however, disruption of the inner plexiform layer was observed in the ventral retina. These data provide strong evidence that proper *pax2a* function is critical in the developing zebrafish embryo. Prolonged viability in the $pax2a^{D9/D9}$ animals indicates that these three amino acids are not critical for renal development, but may be eye specific.

318. Wnt signaling and Sox2 regulate the contribution of midline progenitors to the notochord and floor plate of zebrafish embryos. ***Richard H Row***, *Benjamin L Martin.* Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY.
The notochord and floor plate are crucial signaling centers during early embryogenesis. In vertebrate embryos the notochord runs through the center of the trunk and tail and provides structural support, in addition to producing the morphogen Sonic Hedgehog. The floor plate of the neural tube consists of the ventral-most cells of that structure and participates in neural tube patterning and axonal pathfinding. As the body elongates during segmentation stages a pool of midline progenitor cells contributes to both of these tissues; these progenitors are maintained immediately dorsal to the posterior end of the notochord through the end of segmentation. Previous studies have shown the effects of extracellular signals on these progenitors during gastrulation but it is unknown whether they retain plasticity after gastrulation completes. We use transgenic zebrafish to cell autonomously activate or block different signaling genes, including Wnt signaling and *sox2*. The power of this approach lies in the fact that the transgenes are inducible by heat shock, so embryogenesis can proceed normally until the stage of interest. We also take advantage of the optical transparency of zebrafish embryos and the ability to generate chimeric embryos by cell transplantation. Transgenic cells can be transplanted into wild-type embryos such that they contribute to the midline progenitor pool, and signaling pathways can subsequently be manipulated by inducing expression of the transgene. Wnt signaling has already been shown to be crucial in patterning a different population of progenitor cells in the extreme posterior of zebrafish embryos. When Wnt signals are blocked in those cells they adopt only neural fates instead of contributing to both neural and somitic tissues. We demonstrate that midline progenitors retain the ability to adopt either fate through the end of segmentation, and that Wnt signaling is required for the notochord fate. We find that a crucial function of Wnt signals is to repress production of the transcription factor Sox2, which we show specifies the neural (floor plate) fate in these cells.

319. Cyp26 Enzymes Are Required within the Anterior Lateral Plate Mesoderm to Balance Cardiac and Vascular Lineages. ***Ariel Rydeen***[1,2,3], *Joshua Waxman*[2,3]. 1) Molecular Developmental Biology Graduate Program, University of Cincinnati College of Medicine; 2) The Heart Institute and Molecular Cardiovascular Biology Division; 3) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.
Normal heart development requires appropriate levels of retinoic acid (RA) signaling as too much or too little can be teratogenic. One way that RA signaling levels are moderated is by Cyp26 enzymes, which metabolize RA into easily degraded derivatives. Previous studies using mouse knockouts have shown that loss of Cyp26a1 or both Cyp26a1 and Cyp26c1 (referred to here as Cyp26 deficient) leads to vascular and cardiac defects. However, the mechanisms underlying these defects in Cyp26 deficient embryos have not been addressed. We have found that in zebrafish, Cyp26a1 and Cyp26c1 are expressed in the anterior lateral plate mesoderm (ALPM) predominantly overlapping with vascular progenitors and not cardiac progenitors. Although singular knockdown of Cyp26a1 or Cyp26c1 does not overtly affect cardiovascular development, Cyp26 deficient embryos have increased atrial cells and reduced cranial vasculature cells. Within the ALPM, Cyp26 deficient embryos exhibit an anterior shift in the expression of cardiac progenitor markers and a corresponding truncation of the adjacent anterior vascular progenitor markers. We further examined the ALPM lineages by performing fate-mapping in Cyp26 deficient embryos and found that, in addition to an anterior shift, the atrial progenitor frequency was significantly increased while the vascular progenitor frequency was significantly decreased, suggesting that there is an expansion of the atrial progenitors at the expense of the ventricular progenitors. Surprisingly, in a series of complementary blastula transplantation experiments, we found that both gain and loss of Cyp26 enzyme expression can have cell non-autonomous consequences on the local environment. Therefore, our results suggest that Cyp26 enzymes are required within the ALPM to limit local RA signaling levels, which is necessary for proper placement of progenitor boundaries to balance the cardiac and vascular lineages.

320. DNA methylation: Investigating the function of DNA methylation in zebrafish embryonic and juvenile development. ***Catherine Scahill***[1], *Richard Clark*[1], *Richard White*[1], *Neha Wali*[1], *Christopher Dooley*[1], *John Collins*[1], *Julian Peat*[2], *Ross Kettleborough*[1], *Wolf Reik*[2], *Derek Stemple*[1], *Elisabeth Busch-Nentwich*[1]. 1) Wellocme Trust Sanger Inst, Cambridge, United Kingdom; 2) The Babraham Institute, Cambridge, CB22 3AT, United Kingdom.

DNA methylation is widely regarded as an epigenetic mechanism that regulates gene expression, and the presence of methylation marks is associated with gene silencing. Our aim is to investigate the functional consequences of loss of DNA methyltransferases, the enzymes that catalyse DNA methylation, and of the tet oncogenes, which encode enzymes that catalyse the oxidation of 5-methylcytosine leading to DNA demethylation, in zebrafish development. Using a high-throughput phenotyping pipeline, we are screening loss of function alleles of the DNA methyltransferase and tet oncogenes during the first five days of development and are assessing whether they are juvenile lethal. We will use DeTCT (Differential expression Transcript Counting Technique) to analyse transcriptional changes in the mutants compared to siblings and associate these data with any morphological phenotypes and changes in methylation state determined by whole genome bisulphite sequencing.

321. Status of the European Zebrafish Resource Center (EZRC). *Robert Geisler, Nadine Borel, Tanja Both,* ***Jana Maier****, Mathias Teucke, Wolf-Gerolf Thies, Olivier Armant, Eduard Gursky, Ravindra Peravali, Uwe Strähle.* European Zebrafish Resource Center, Institute of Toxicology and Genetics, Karlsruhe Institute of Technology (KIT), Germany.
A severe limitation of zebrafish research in Europe has been the lack of a centralised screening and stock center. We have therefore established the European Zebrafish Resource Center (EZRC) which provides a permanent repository for mutant and transgenic zebrafish lines produced in Europe, mainly as frozen sperm. Through our website http://ezrc.kit.edu we have begun distribution of more than 2,000 published alleles produced in large-scale screens at the MPI EB, Tübingen and 10,000 alleles from the ongoing genome-wide knockout experiment at the Sanger Institute, in addition to transgenes and wildtype lines. The EZRC also provides plasmids and sequencing services and hosts training courses for persons carrying out experiments in fish. As a central hub of the ZF-HEALTH EU project we will host genetic screens and chemical screens for guest researchers, focusing on the Sanger mutants.

322. Addressing the role of the WTX / AMER protein family in zebrafish. ***Andreas Grosse****, Birgit Perner, Christoph Englert.* Molecular Genetics, Leibniz Institute for Age Research (FLI), Jena, Germany.
The AMER (APC membrane recruitment) protein family consists of three members. AMER1 is also named WTX and is encoded by an X-linked gene that is mutated in Wilms tumor, a form of pediatric kidney cancer. In addition, germline mutations in WTX were detected in patients suffering from OSCS (Osteopathia striata with cranial sclerosis), a sclerosing bone disorder. These observations suggest that in addition to its tumor suppressor function WTX acts as an essential regulator during embryonic development. Almost nothing is known about the physiological role of AMER2 and AMER3. In order to gain insights into the molecular basis of WTX-associated diseases as well as the function of the other AMER family members we are using the zebrafish as a model. Like in higher vertebrates zebrafish Amer1 and Amer2 display more similarity to each other than either of them with Amer3. Regarding the temporal expression pattern (from 4 cell stage to 3 days post fertilization) we found stronger expression of amer1 and amer2 at very early stages. In contrast amer3 is expressed at later stages of development. We have employed TALENs to introduce mutations close to the ATG start codon of zebrafish wtx. We have generated several independent mutant alleles, however, none of them is associated with phenotypic alterations. Cell culture data suggest that this is caused by usage of additional in-frame start codons, which are localized downstream of the targeting site. We are now using an approach in which we simultaneously employ two TALEN pairs spanning 0.5 to 1.2 kb of the wtx locus. Recent analysis shows that we could successfully use this technology to generate deletions in wtx. We have also employed this technique to generate amer2 and amer3 mutant lines. We are currently generating compound heterozygous as well as homozygous lines. Those lines will be helpful to gain insights into the molecular pathways that are regulated by members of the AMER family.

323. High throughout genotyping of SNPs and CRISPR induced INDELs using KASP technology. ***Amanda L Hall****, Zsofia Puztai, Christopher Dooley, Catherine Scahill, Samantha Carruthers, Nicole Staudt, Elisabeth Busch-Nentwich, Derek L. Stemple, Ross N W Kettleborough.* Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom.
The Zebrafish Mutation Project (ZMP) aims to generate a knock out allele for every protein coding gene in Zebrafish and describe any associated phenotypes caused by these alleles. Zebrafish are mutagenized with ENU and induced mutations in each individual are detected using exome pulldown and NGS. To maximise screening a two-step multi-allelic phenotyping pipeline has been set-up; Firstly exome sequenced F1 males are outcrossed generating F2s, which are then incrossed and the resulting F3 embryos screened. Phenotypically wild-type embryos are genotyped. Secondly any potentially causative alleles are noted and carriers for these are incrossed and their F3 embryos are phenotyped over the first 5 days of development. Mutations are confirmed with KASP genotyping (LGC Genomics, formerly Kbioscience), an allele specific amplification of target SNPs using a fluorescence (FRET) based system. Specific primers are designed for both the mutant and wild-type SNP. Amplification is short and results are scanned after two hours with the genotypes determined by their fluorescence profile. To increase our capacity we have worked this up to genotype across 384 well plates in a reaction volume of 4ml allowing us to fully automate the process and efficiently genotype whole families in a very short time frame. To complement the exome pulldown and sequencing approach we are using the crispr/Cas9 system to generate targeted INDELs. This system will be used to generate mutations in specific genes which are not covered using ENU mutagenesis, and resulting INDELs can also be genotyped in a high throughput way using KASP. The genotyping methods we have developed in conjunction with LGC has led to highly efficient genotyping, saving time and effort in identification of mutant carriers.

324. Generating zebrafish knockout mutants for a DSCR gene using TALEN. *Hyun-Ju Cho[1,2], Se-youl Yang[1,2], Kwon Yu[1,2],* ***Jeong-Soo Lee***[1,2]. 1) Bionano center, KRIBB, Daejeon, 305-333, Korea; 2) Functional Genomics Dept., University of Science and Technology (UST), Daejeon 305-333, Korea.
Down syndrome (DS) is one of the most common human genetic disorders caused by trisomy of chromosome 21. People with DS display characteristic facial features, mild to severe mental retardation, congenital heart malfunction, and increased risk of leukemia. DSCR (Down Syndrome Critical Region) is the minimal segment of chromosome 21 shared by DS patients and thought to contain a gene(s) critical for

such abnormalities. DYRK1A, located in DSCR, is a non-receptor Ser/Thr kinase that phosphorylates signaling molecules implicated in cell proliferation, migration, and survival, and suggested to be a strong candidate gene responsible for diverse DS phenotypes. In order to study DYRK1A function using zebrafish as a model system, we identified zebrafish homologs and examined their expression patterns mainly found in the nervous and vascular systems of the head and trunk during embryogenesis. We generated loss-of-function mutants for one of the homologs using TALEN (Transcription activator-like effector nuclease) technique. We identified three founder fish carrying mutations after screening 65 fish (3/65=4.61% germline transmission rate). A detailed characterization of phenotypes of these mutant fish will shed light on understanding diverse roles of DYRK1A during development.

325. CRISPR/Cas9-mediated genome modifications in zebrafish. ***Satoshi Ota***[1,2], *Yu Hisano*[1], *Atsuo Kawahara*[1,2]. 1) Laboratory for Cardiovascular Molecular Dynamics, Riken Quantitative Biology Center, Suita, Osaka, Japan; 2) Laboratory for Developmental Biology, Center for Medical Education and Sciences, Graduate School of Medical Science, University of Yamanashi, Chuo, Yamanashi, Japan.
The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system is a powerful tool in genome editing. CRISPR/Cas9 consists of the nuclease Cas9 and guide RNA (gRNA) that interacts with target genomic locus; the Cas9-gRNA complex induces a double strand DNA break at the target site, reading to the frameshift-mediated gene disruption. This characteristic feature of CRISPR/Cas9 enables us to disrupt multiple genes by multiple gRNAs/Cas9 injection in zebrafish. However, it is still unclear whether insertion and/or deletion (indel) mutations of multiple genes induced by multiple gRNAs plus Cas9 are heritable. We designed 5 gRNA against four genes (*golden*/*gol*, *tyrosinase*/*tyr*, *sphingosine-1-phosphate receptor 2*/*s1pr2* and *spns2*). Both *gol* and *tyr* are involved in pigment formation, while *s1pr2* and *spns2* are involved in cardiac development. When 5 gRNAs (gol-gRNA, tyr-gRNA, s1pr2-gRNA, spns2-gRNA1 and spns2-gRNA2) plus Cas9 were injected into zebrafish embryos, we observed two distinct phenotypes, two hearts phenotype and hypopigmentation of skin melanophores and retinal pigment epithelium in F0 embryos. We simultaneously detected chromosomal deletion and inversion between the two *spns2* gRNA target sites in F0 embryos. From analyses of F1 embryos derived from F0 founders, we found that CRISPR/Cas9-induced multiple genome modifications including indel mutations and chromosomal deletion were heritable, demonstrating that the CRISPR/Cas9 is suitable for multiple genome modifications in zebrafish.

326. Evaluating the efficiency of genome modification using the CRISPR-Cas9 system in zebrafish. ***Yan Pi***[1], *Keji Jiang*[1], *Zhiqiang Dong*[1], *Stanley Qi*[2], *Su Guo*[1]. 1) Bioengineering and Therapeutic Science, University of California, San Francisco, San Francisco, CA; 2) Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA.
The CRISPR-Cas9 system has been shown to be highly efficient in genome editing in many different systems including zebrafish. Here we evaluate its efficiency by comparing different Cas9 molecules and different designs of small guide (sg) RNAs. We also test different concentrations of cas9 RNA, protein, and sgRNA concentrations. It is our hope that further improvement of the efficiency will make the technology useful for generating knock-in DNA cassettes at defined loci in the future.

327. CRISPR MultiTargeter in zebrafish: a bioinformatics tool to find common and unique CRISPR guide RNA targets of any type in a set of similar sequences. ***Sergey V. Prykhozhij***, *Vinothkumar Rajan*, *Jason N. Berman.* Pediatrics (Aquatics Laboratory), IWK Health Centre, Halifax, NS, Canada.
The genome modification toolbox has been dramatically enriched by the discovery of CRISPR/Cas systems consisting of clustered regularly interspaced palindromic repeats (CRISPR) in the genome and CRISPR-associated system genes (Cas) in bacteria. The Type IIB CRISPR/Cas9 system has been successfully used in zebrafish. In this system, combining CRISPR guide RNA (gRNA) targeting a defined sequence and the Cas9 enzyme generates a sequence-specific nuclease, whose activity results in small deletions or insertions. Moreover, precise insertion of larger DNA inserts has been shown at the sites targeted by gRNAs and Cas9. In all CRISPR/Cas system applications, computational design of potential genomic targets is of critical importance. Several tools are available for the design of gRNA targets unique in the genome. However, ability to find gRNA targets common for several similar sequences or unique to each of these sequences may be advantageous. To have a tool to achieve this aim in the zebrafish and other species and for any type of CRISPR/Cas system, we developed CRISPR MultiTargeter software. Examples of similar DNA sequences in question are duplicated genes, common in zebrafish, and alternative exons in a gene. Thus, we implemented 1) a basic gRNA target search in input sequences and searches of common and unique gRNA targets in 2) a set of input sequences; 3) a set of transcripts of a single gene; or 4) a set of similar genes or transcripts. Generation of common gRNA targets for duplicated genes can simplify the design of multi-gene mutational targeting experiments. Design of unique targets in alternative exons is a helpful use of the program since finding such targets manually is time-consuming. This application will facilitate functional genomic studies of transcript isoforms. In addition to making this tool available online, we plan to measure the efficiencies of mutation induction at common target gRNA sites in similar genes in zebrafish. Overall, this program provides a unique in silico interface that will enhance use of CRISPR/Cas technology for the zebrafish community.

328. The long-range regulation of transcription factor gene ARX is affected by genomic rearrangements in patients with intellectual disability. *Minaka Ishibashi*[1], *Elizabeth Manning*[1], *Cheryl Shoubridge*[2], *Thomas Hawkins*[3], *Thomas Mueller*[4], *Jozef Gecz*[5], *Bernard Peers*[6], *Thomas S Becker*[1], ***Silke Rinkwitz***[1]. 1) BMRI, University of Sydney, Camperdown, NSW, Australia; 2) School of Paediatrics, University of Adelaide, Adelaide, SA 5006, Australia; 3) Department of Anatomy and Developmental Biology, UCL, London WC1E 6BT, UK; 4) Division of Biology, Kansas State University, Manhattan, KS 66502, Kansas, United States; 5) Genetics and Molecular Pathology, Women's and Children's Hospital, Adelaide, SA 5006, Australia; 6) Giga-Research, University of Liège, B-4000 Sart-Tilman, Belgium.
Several studies have linked mutations in the protein coding part of the transcription factor gene ARX, located on the X chromosome with severe forms of intellectual disability (ID) or epilepsy. Mutations in surrounding non-coding sequences, in contrast, are correlated with milder forms of ID and related phenotypes. These latter results point to the importance of gene regulatory sequences for the etiology of neurological defects. To shed light on the molecular mechanisms affecting ARX expression, we studied the regulation of the gene.

Specifically, our study identifies the long-range regulatory domain of ARX and maps new duplications of this region in patients with ID. Furthermore, using zebrafish as a readout model, we describe five brain region specific ARX enhancers. Using these lines as tools for analyzing gene function in the brain, we discover a two enhancer dependent negative autoregulatory feedback mechanism. This mechanism leads to enhanced Arx transcription in subregions of the expression domain when arx protein synthesis is inhibited. The use of reporter lines with fluorescent labeling in developing brain regions positively regulated for ARX/arx in arx-inhibition experiments revealed neuronal and wiring deficits in the ventral thalamus and ventral telencephalon. We present a hypothetical model that explains how variation in long-range gene regulation alters the expression levels of a target gene with effects on the etiology of a neurological disease phenotype.

329. ZebrafishMine: a new way to access ZFIN data. ***Leyla Ruzicka****, Sierra Moxon, Anne Eagle, Doug Howe, Prita Mani, Ryan Martin, Christian Pich, Monte Westerfield.* The Zebrafish Model Organism Database (ZFIN), Institute of Neuroscience, University of Oregon, Eugene, OR.
ZebrafishMine (http://zebrafishmine.org), powered by the data warehousing system InterMine (http://intermine.org), offers new and powerful ways to explore ZFIN's data.
ZebrafishMine can be searched using individual terms, or by using lists of biological entities (such as genes, alleles, or Gene Ontology terms). Complex searches can be custom-built using a sophisticated "query builder". ZebrafishMine also includes sets of predefined complex search templates, which can be modified. Search results can be sorted and filtered. Results can be saved and downloaded in a variety of file formats.
In addition to weekly updates of ZFIN data, ZebrafishMine also includes computed homology data from the Panther data source (http://pantherdb.org). New data types will continue to be added.
ZebrafishMine provides web services for programmatic data access to its features, with client library support for Perl, Python, Ruby, and Java.

330. High-Content Screening in Zebrafish Embryos Identifies Butafenacil as a Potent Inducer of Anemia. ***Jessica K Leet****, Casey D Lindberg, Luke A Bassett, Gregory M Isales, Kryslte L Yozzo, Tara D Raftery, David C Volz.* Environmental Health Sciences, University of South Carolina, Columbia, SC.
Targeted high-throughput assays are needed to better predict morphologic and functional effects of chemicals on cardiovascular development. Using transgenic zebrafish (*fli1:egfp*) we recently developed and optimized a 384-well high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at non-teratogenic concentrations. Within this assay, automated image acquisition procedures and custom image analysis protocols are used to quantify body length, heart rate, circulation, pericardial area, and intersegmental vessel area within individual live embryos exposed from 5-72 hours post-fertilization. After ranking acute toxicity data generated from the U.S. Environmental Protection Agency's (EPA's) zebrafish teratogenesis assay, we screened ~10% of the most potent chemicals within EPA's ToxCast™ Phase-I library in concentration-response format (0.05-50 mM) using this HCS assay. Based on this screen, we identified butafenacil as a potent inducer of anemia, as exposure to 0.39-3.125 mM butafenacil completely abolished arterial circulation in the absence of effects on all other endpoints evaluated. Butafenacil is an herbicide that inhibits protoporphyrinogen oxidase (PPO) - an enzyme necessary for heme production in vertebrates. Using o-dianisidine staining, we revealed that severe butafenacil-induced anemia in zebrafish was due to a complete loss of hemoglobin following exposure during early development. Therefore, six other PPO inhibitors within the ToxCast™ Phase-I library were screened to determine whether anemia represents a common adverse outcome for these herbicides. Developmental exposure to only one of these PPO inhibitors - flumioxazin - resulted in a similar phenotype as butafenacil, albeit anemia induced by flumioxazin was not as severe as butafenacil. Overall, this study highlights the utility of this assay for (1) screening chemicals for targeted effects on cardiovascular function and (2) prioritizing chemicals for future hypothesis-driven and mechanism-focused investigations within zebrafish and mammalian models.

331. High-content screening assay for identification of chemicals impacting spontaneous activity in zebrafish embryos. ***Tara D. Raftery****, Gregory M. Isales, Krystle L. Yozzo, David C. Volz.* Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC USA 29208.
Although cell-based assays exist, rapid and cost-efficient high-content screening (HCS) assays within intact organisms are needed to support prioritization for developmental neurotoxicity (DNT) testing in rodents. During zebrafish embryogenesis, spontaneous tail contractions occur from late-segmentation (~19 hours post-fertilization, hpf) through early-pharyngula (~29 hpf) and represent the first sign of locomotion. Using transgenic zebrafish (*fli1:egfp*) that stably express eGFP beginning at ~14 hpf, we have developed and optimized a 384-well-based HCS assay that quantifies spontaneous activity within single zebrafish embryos after exposure to test chemicals in a concentration-response format. Following static exposure of one embryo per well from 5 to 25 hpf, automated image acquisition procedures and custom analysis protocols were used to quantify total body area and spontaneous activity in live embryos. Survival and imaging success rates across control plates ranged from 87.5-100% and 93.3-100%, respectively. Using our optimized procedures, we screened 16 chemicals within the US EPA's ToxCast™ Phase-I library, and found that exposure to abamectin and emamectin benzoate - both potent avermectins - abolished spontaneous activity in the absence of gross malformations. Overall, compared to existing locomotion-based zebrafish assays conducted later in development, this method provides a simpler discovery platform for identifying potential developmental neurotoxicants.

332. Developing FingRs for Monitoring of Synapses in the Embryonic Zebrafish Brain. ***J.H. Son***[1], *T.M. Dahl*[2], *T.J. Stevenson*[1], *J.L. Bonkowsky*[1]. 1) Pediatrics, University of Utah, Salt Lake City, UT; 2) Department of Neurobiology & Anatomy, University of Utah, Salt Lake City, UT.
The ability to visualize endogenous proteins in living neurons provides a powerful tool to study neuronal structure and function. Recently, recombinant antibody-like proteins, termed Fibronectin intrabodies (FingRs), have been developed and tested to visualize endogenous synaptic proteins: postsynaptic density protein 95 (PSD-95) and Gephyrin (GPHN), in order to visualize excitatory and inhibitory synapses in dissociated and brain slices (Gross et al., 2013). Herein, we have tested whether PSD-95 and GPHN can be visualized at excitatory and inhibitory synapses using the FingR approach in developing zebrafish brains in order to study dopaminergic and serotonergic connectivity. Our goal is to use FingRs to characterize normal synaptic connectivity, and to explore the effects of disease processes on synapses. We have generated and tested FingRs for PSD95 and GPHN. First, we found that PSD95.FingR-GFP and GPHN.FingR-GFP can be expressed and label neurons and neurites. Next, to limit over-expression of the FingRs which prevents precise synaptic labeling, we generated constructs in which expression is regulated by an inhibitory zinc-finger transcriptional control system. We injected and tested UAS:PSD95.FingR-egfp-CCR5TC and UAS:GPHN.FingR-mKate2-IL2RGTC in Tg(otpb.A:Gal4), labeling dopamine neurons, and Tg(pet1:Gal4), labeling serotonin neurons. We are confirming specificity of labeling using standard immunohistochemistry. Together with other approaches (e.g. mGRASP) we expect these tools to provide novel means for characterization of synaptic connectivity development in the CNS.

333. Characterization and role of *id2a* in zebrafish liver development. ***Mehwish Khaliq***, *Juhoon So, Donghun Shin*. Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA 15260, USA.
To regulate various developmental processes such as myogenesis, neurogenesis, and cardiogenesis, helix-loop-helix (HLH) proteins act as transcriptional repressors or activators. A subfamily of HLH proteins, inhibitor of differentiation (ID), lacks the basic DNA binding domain; instead, ID proteins act in a dominant negative fashion to sequester bHLH transcription factors and regulate cell proliferation, differentiation and survival. In vertebrates, there exist four members of the ID protein family (ID1-4). Specifically, using zebrafish as a model organism, we chose to focus on *id2a*, a paralog of the *Id2* gene, to determine its functional role and mechanism of action, including binding partners, during liver development and regeneration. During liver regeneration following acute liver injury in the zebrafish hepatocyte-specific ablation model, *id2a* is upregulated. Using fluorescent whole-mount in situ hybridization, we show *id2a* expression in hepatoblasts at 30 hours post fertilization (hpf) and biliary epithelial cells (BECs) at 48 hpf; *id2a* expression persists in the BECs even at 5 dpf. Knockdown of *id2a* with morpholino antisense oligos (MO) results in a reduced liver size as indicated by *in situ* with hepatoblast markers (*prox1*, *hhex* and *myca*) at 36 hpf and 48 hpf and hepatocyte markers (*fabp10a* and *cp*) at 48 hpf and 72 hpf. We observed decreased proliferation and increased apoptosis in the liver at 40 hpf, as indicated by 5-ethynyl-2'-deoxyuridine (EdU) incorporation and TUNEL assay, respectively. For *id2a* overexpression analyses, we generated a transgenic line, *Tg(hsp70:mCherry-T2a-id2a)*; upon heat-shock at 22 hpf or 30 hpf, we observed no liver development phenotype at 30 hpf or 48 hpf, respectively. In the future, we plan on studying later liver developmental phenotypes using homozygous *id2a* mutants generated by TALEN mutagenesis. We will also examine whether liver regeneration is compromised in the *id2a* mutants using various liver injury models, including acetaminophen treatment and hepatocyte-specific genetic ablation model. Furthermore, we will examine the involvement of signaling pathways, such as BMP and Wnt, in regulating *id2a* activity and function during liver development and regeneration.

334. The ribosomal RNA processing gene nucleolar protein 9 (nol9) is essential for normal exocrine pancreas development in zebrafish. ***Laure Lam Hung***[1], *Steve Harvey*[1], *Inës Barroso*[1,2], *Derek Stemple*[1]. 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Institute of Metabolic Science, Cambridge, United Kingdom.
Zebrafish is an excellent model organism for the study of pancreatic development and diseases. The Zebrafish Mutation Project (ZMP) aims to create a loss of function allele in every protein-coding gene in the zebrafish genome, and to analyse these mutant zebrafish for morphological differences. One such zebrafish was a mutant of nucleolar protein 9 (nol9), *nol9*^{sa1022} that was found to be associated with a pancreas phenotype. The Nol9 protein is known to be involved in processing of 28S rRNA of the large ribosomal subunit. The main aim of this project is to determine the role of nol9 in pancreas development by studying the *nol9*^{sa1022} mutant. The characterisation of *nol9*^{sa1022} mutant revealed that the pancreas, liver and intestine failed to develop properly after 3 days post fertilisation and that this was at least in part due to impaired cell proliferation. The development of all the other organs appeared unaffected. Interestingly, *las1l*^{sa674}, a zebrafish mutant allele of a Nol9-interacting protein with similar function, was found to exhibit digestive organ defects. An mRNA expression analysis of *nol9*^{sa1022} mutant revealed an up-regulation of genes belonging to the Tp53-signalling pathway. However, the genetic loss of Tp53 did not appear to suppress the pancreatic defects of the *nol9*^{sa1022} mutant suggesting the involvement of a Tp53-independent mechanism. The functional group analysis revealed that upregulated genes in *nol9*^{sa1022} mutants were enriched for functions in rRNA processing, ribosome biogenesis, and translation, consistent with the known function of the protein. Altogether, these findings suggest that the *nol9*^{sa1022} mutant can be used as a model to study the mechanisms by which impaired development of digestive organs arises in rRNA processing mutants and ribosomopathies.

335. *Fibroblast growth factor 24* is required for early somatic gonad development in zebrafish. ***Dena M. Leerberg***[1], *Kaori Sano*[2], *Lan-Uyen S. Nguyen*[1], *Bruce W. Draper*[1]. 1) Molecular and Cellular Biology, University of California, Davis, Davis, CA; 2) Materials and Life Sciences, Sophia University, Tokyo, Japan.
Germ cells contain the only genetic material inherited by our offspring. These cells are protected and nurtured by specialized somatic cells of the gonad. Signaling between germ cells and somatic gonad cells is also known to be essential for sex determination and gametogenesis. The mechanisms mediating the development of the somatic gonad in vertebrates during the transition from the sexually bipotential state to the sex-specified state are well defined. In contrast, far less is known about the genes acting earlier during formation of the undifferentiated

gonad primordium. We recently discovered that a mutation in the zebrafish Fgf ligand, *fgf24*, results in the rapid loss of germ cells during larval development at a time prior to gonad sexual differentiation, and most mutant adults are sterile. We have determined that *fgf24* is expressed in somatic gonad cells in larvae. We therefore hypothesize that Fgf24 is produced by somatic gonad cells and functions primarily to promote somatic gonad development and that the loss of germ cells in *fgf24* mutants is a secondary consequence of a somatic gonad defect. To test this hypothesis, we first determined by genetic mosaic analysis that the ligand is required to be produced by somatic but not germ cells. Furthermore, to test whether the Fgf24 ligand is specifically produced by somatic gonad cells rather than other somatic tissues, we have developed a novel method for producing genetic mosaic somatic gonads. Next, we compared the expression of several known or newly identified genes that are expressed in the somatic gonad at 10 and 12 days post fertilization between wild-type and *fgf24* mutants, a timepoint that precedes loss of germ cells. Our results from this analysis indicate that, in most cases, expression of these genes can be detected in wild-type but not in *fgf24* mutant gonads. Together these data strongly support a role for Fgf24 signaling in the development of the somatic component of the early bipotential gonad.

336. Zebrafish nephrogenesis is regulated by interactions between retinoic acid, *mecom*, and Notch signaling. ***Yue Li**, Christina Cheng, Valerie Verdun, Rebecca Wingert.* Dept Biological Science, Univ Notre Dame, Notre Dame, IN.
The zebrafish pronephros provides a conserved model to study kidney development, in particular to delineate the poorly understood processes of how nephron segment pattern and cell type choice are established. Zebrafish nephrons are divided into distinct epithelial regions that include a series of proximal and distal tubule segments, which are comprised of intercalated transporting epithelial cells and multiciliated cells (MCC). Previous studies have shown that retinoic acid (RA) regionalizes the renal progenitor field into proximal and distal domains and that Notch signaling later represses MCC differentiation, but further understanding of these pathways has remained unknown. The transcription factor *mecom* (*mds1/evi1 complex*) is broadly expressed in renal progenitors, and then subsequently marks the distal tubule. Here, we show that *mecom* is necessary to form the distal tubule and to restrict both proximal tubule formation and MCC fate choice. We found that *mecom* and RA have opposing roles in patterning discrete proximal and distal segments. Further, we discovered that RA is required for MCC formation, and that one mechanism by which RA promotes MCC fate choice is to inhibit *mecom*. Next, we determined the epistatic relationship between *mecom* and Notch signaling, which limits MCC fate choice by lateral inhibition. Abrogation of Notch signaling with the *g*-secretase inhibitor DAPT revealed that Notch and *mecom* did not have additive effects in blocking MCC formation, suggesting that they function in the same pathway. Ectopic expression of the Notch signaling effector, Notch intracellular domain (NICD), rescued the expansion of MCCs in *mecom* morphants, indicating that *mecom* acts upstream to induce Notch signaling. These findings suggest a model in which *mecom* and RA arbitrate proximodistal segment domains, while MCC fate is modulated by a complex interplay in which RA inhibition of *mecom*, and *mecom* promotion of Notch, titrates MCC number. Taken together, our studies have revealed several essential and novel mechanisms that control pronephros development in the zebrafish.

337. The *tbx2a/b* transcription factors direct pronephros segmentation while interplay between *tbx2b* and Notch controls corpuscle of Stannius formation in zebrafish. ***Yue Li**, Kristin Springer, Rebecca Wingert.* Dept Biological Science, Univ Notre Dame, Notre Dame, IN.
The zebrafish embryonic pronephros is an excellent model of kidney development, as it possesses remarkable conservation with other vertebrate nephrons. The zebrafish pronephros is segmented into functionally distinct regions including the proximal convoluted and straight tubule (PCT, PST), distal early and late (DE, DL) tubule, and a pronephric duct (PD). In addition, endocrine cells called the corpuscle of Stannius (CS) are interpolated between the DE and DL segments. How nephron pattern is established and how the CS is formed during nephrogenesis remains intriguing. Previous research in *Xenopus* has identified the transcription factor *Tbx2* as a key regulator in defining the pronephric territory. Using whole mount *in situ* hybridization, we found that transcripts encoding the zebrafish *tbx2* orthologues *tbx2a* and *tbx2b* were spatially restricted to the distal renal progenitors during pronephros formation. To elucidate the function of these *tbx2* genes in zebrafish nephrogenesis, morpholino knockdown studies were performed. *tbx2a* and *tbx2b* single and double morphants exhibited a modest expansion in the proximal segments accompanied by a reduction in the distal domains, indicating that these genes have redundant roles segment patterning. Noticeably, *tbx2b* morphants formed significantly larger CS clusters, as shown by the elevated expression of the marker *stc1*. Further, in preliminary studies we identified expression of the Notch pathway components *notch3* and *her9* in the developing CS. To study the possible link between *tbx2b* and Notch, DAPT treatment was used to block Notch activity in wild types and *tbx2b* morphants. DAPT treatment resulted in moderate CS expansion in wild types, while DAPT induced further enlarged CS clusters in *tbx2b* morphants. Supporting this result, ectopic activation of Notch signaling in *Tg(hsp70::Gal4; UAS::NICD)* led to a reduced CS post heat-shock induction. Taken together, these data suggest the new model that *tbx2a/b* activities mitigate nephron segmentation while cooperation of *tbx2b* and Notch signaling regulates CS formation in the zebrafish pronephros.

338. Differential expression of tight junction genes during zebrafish pronephros development. ***Robert A. McKee**, Gary F. Gerlach, Jonathan Jou, Christina N. Cheng, Rebecca A. Wingert.* Biological Sciences, Notre Dame, Notre Dame, IN.
The kidney is comprised of specialized epithelial tubules, termed nephrons, which are regionalized into a series of proximal and distal segments. Each segment exhibits unique combinations and expression levels of ion channels and transporter proteins, enabling them to facilitate intracellular recovery and secretion of particular solutes. Kidney function is also contingent on the ability of nephrons to regulate the paracellular movement of molecules. Paracellular permeability is governed by interactions between proteins at the apical tight junctions of neighboring renal epithelial cells. Furthermore, mutations in these proteins have been shown to lead to defective kidney function such as hypomagnesemic hypercalciuria. Following nephron damage, proper epithelial regeneration and kidney functionality relies on the re-establishment of cell-cell contacts, mediated in part by tight junctions. The following study implemented a modified whole mount in situ hybridization approach to analyze renal expression of claudin (cldn) 15a, cldn8, occludin (ocln) a, oclnb, and three tight junction proteins/zona occludens (tjp) in zebrafish. By 24 hours post fertilization (hpf), cldn15a and cldn8 demarcated the proximal and distal segments, respectively. Interestingly, the expression domains of these cldns partially overlapped during earlier stages of development. The

tjp transcripts showed a dynamic expression pattern, present throughout the whole pronephros at the 16 somite stage (ss), with a gradual restriction to the distal regions by 36hpf. The presence of ocln transcripts were first detected later in development at approximately 20ss. oclna was expressed in the whole tubule with a rapid restriction to the cloaca by 28ss. oclnb was expressed at 20ss in the distal late segment and collecting duct. Taken together, these data provide a detailed spatiotemporal expression map of the tight junction transcripts present in the renal progenitors during nephrogenesis. Knowledge of these expression domains can aid in characterization of nephron damage and regeneration after insult, as well as contribute to the assessment of nephron defects in a novel panel of zebrafish kidney mutants recently isolated in our lab.

339. Forward Screen for Genetic Analysis of Podocyte Development Using Zebrafish. ***Rachel Miceli****, Paul T. Kroeger Jr., Gary F. Gerlach, Shahram J. Poureetezadi, Rachel Bounds, Jennifer Cihlar, Annemarie Fox, Michael McKernan, Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
The vertebrate kidney is composed of functional units called nephrons. The zebrafish embryonic kidney is made up of two nephrons that share a common blood filter, and provides a simplified genetic model to identify genes essential for nephron cell type development. Podocytes are specialized epithelial cells that derive from renal progenitors and become crucial components of the filter. Mature podocytes are characterized by cellular extensions, called foot processes, which project from their basal surface. These foot processes interdigitate with neighboring podocytes and form cell-to-cell junctions called the slit diaphragm. The mechanisms that elaborate podocyte specification and differentiation are not well understood. Previous study of *lightbulb (lib)* mutant embryos, which have a mutation in *aldehyde dehydrogenase 1a2 (aldh1a2)* that is required for retinoic acid (RA) biosynthesis, showed that RA is essential for podocyte development. *lib* mutants form dramatically reduced numbers of podocytes, which can be rescued by exogenous RA treatment. To find additional components required for podocyte ontogeny, we performed an F2 screen to identify haploid embryos with alterations in podocyte number. To date, five recessive mutations affecting podocyte development have been isolated and characterized. ND154, ND172 and ND233 have a severe loss of podocytes, while ND298 and ND325 have a moderate reduction in podocyte number based on expression levels of the podocyte-specific transcription factor, *wt1b*. Interestingly, ND154 failed to complement *lib* and exogenous RA rescued ND154 podocytes, identifying this as a new *aldh1a2* allele. In contrast, RA addition to ND172 and ND233 mutant clutches was not sufficient to rescue podocyte numbers, suggesting these genes play roles downstream of RA signaling during podocyte development. Ongoing characterization and cloning of our podocyte mutant collection can provide new insights into the gene regulatory networks that are responsible for directing podocyte development that may be applicable to understanding the plethora of podocyte-related kidney diseases.

340. Transcriptomic Analysis of An Induced Ploidy Series in Zebrafish. ***A Hasley****, K Takle, J Heier, F Pelegri.* Genetics, University of Wisconsin-Madison, Madison, WI.
Tolerance of polyploidy (possessing 3 or more complete haploid sets of chromosomes) varies across the tree of life. It appears rare in animals, especially vertebrates, can be associated with tumorigenesis, and is lethal in humans. However, polyploidy is widespread in plants, is part of many fungal life cycles, and is even normal in some mammalian cell-types (e.g. liver cells and megakaryocytes). This raises a question. Why are ploidy changes tolerated in some contexts but not others? Here, we present an ongoing study seeking to identify immediate biological consequences of ploidy change in a vertebrate by comparing the transcriptomes of 1-day-old zebrafish embryos of varying ploidy. These data will point to biological processes impacted by ploidy changes, and help us understand why this phenomenon seems to be generally deleterious in vertebrates. Our preliminary data comparing wildtype diploid and haploid embryos using microarrays yielded some notable results. Genes associated with ribosomes and translation are significantly up-regulated in haploids relative to diploids. This might be in line with studies suggesting that housekeeping genes sensitive to stoichiometry are retained over evolutionary time after polyploidization events. Several Crystalin proteins are also up-regulated in haploids, along with other chaperone proteins. This suggests that haploids may struggle to cope with excess aberrant protein aggregation due to higher concentrations caused by reduced volume of haploid cells. Finally, down-regulation of segmentation and patterning genes may indicate that haploids are experiencing a delay in expression onset of these genes, disrupting development. We are expanding on this experiment. We have developed reliable means of generating tetraploids (which die at 5 dpf.) using heat-shock. We are including transcriptomes of these embryos in our study. We are also adopting RNAseq methodology to improve the quality and reliability of our data. These experiments will allow robust comparison of expression profiles of an induced ploidy series in a model vertebrate, which is rare. This knowledge will deepen our understanding of the specific biological challenges posed to vertebrates by ploidy changes.

341. Retention of ancestral developmental potential for dentition varies between fish lineages. ***David Jandzik***[1,2]*, Sharon R. Aigler*[1]*, Kohei Hatta*[3]*, Kentaro Uesugi*[4]*, David W. Stock*[1]. 1) University of Colorado, Boulder, USA; 2) Comenius University, Bratislava, Slovakia; 3) University of Hyogo, Japan; 4) SPring-8, Japan Synchrotron Radiation Research Institute, Hyogo, Japan.
Dentition in ray-finned fishes was ancestrally widespread throughout the oropharyngeal cavity, with the predominant evolutionary trend being tooth loss in the central region and retention in anterior and posterior ones. Reversal of such tooth loss is rare but has occurred in a number of groups. This asymmetric frequency of tooth loss relative to regain may be explained either by selection for dentition reduction or developmental constraints on the reappearance of lost teeth. We have investigated these possibilities by comparing the effects of expressing tooth initiation signals in the zebrafish, which retains teeth only in the posterior ventral pharynx, and the Mexican Blind Cave Tetra (*Astyanax mexicanus*), which possesses teeth dorsally and ventrally in both the jaw margins and the posterior pharynx. Continuous and ubiquitous transgenic expression of the TNF family ligand Ectodysplasin (Eda) in the zebrafish results in ectopic teeth limited to the dorsal and ventral posterior pharynx, despite the competence to respond to Eda signaling (as indicated by *NFkB:gfp* reporter expression) existing throughout the oropharyngeal epithelium. This result suggests a constraint on regaining central and anterior teeth in the form of evolutionary alteration of pathways in addition to Eda signaling. Conversely, the relative ease of regaining dorsal posterior teeth in the zebrafish suggests selection as the reason that they have never reappeared during the diversification of the order Cypriniformes, to which

this species belongs. Surprisingly, overexpression of Eda in *A. mexicanus* results in the appearance of ectopic teeth in the central oropharynx, both on anterior gill arches as well as several bones of the palate. This result suggests not only that constraints on regaining lost teeth vary among lineages but a potential mechanism for the occasional reappearance of teeth in the central oropharyngeal cavity in ray-finned fish evolution.

342. Gene Loss, Duplication, and Expression Divergence in Vertebrate Glypicans. *Kenneth Kramer[1], Harry Choi[2], Niles Pierce[2],* ***Yun Kee***[3]. 1) Biomedical Sciences Department, Creighton University School of Medicine, Omaha, NE; 2) Department of Bioengineering, California Institute of Technology, Pasadena, CA; 3) Department of Systems Immunology, Kangwon National University, Chuncheon, South Korea.
Glypicans are a family of cell-surface heparan sulfate proteoglycans that mediate multiple cell-cell signaling pathways during embryonic development. To better understand how the 10 zebrafish glypicans might be evolutionarily comparable to the 6 human and mouse glypicans, we cloned the entire glypican family from Callorhinchus milii (elephant shark), Xenopus tropicalis (frog), and Gallus gallus (chicken). Our extensive phylogenetic analysis reveals that early vertebrate glypicans have been both lost in distinct vertebrate lineages and duplicated in zebrafish. By taking advantage of the recently developed multiplexed bioimaging technology using programmable in situ amplification, we compared gene expression between each pair of duplicated zebrafish glypican genes. We observed that expressions of duplicated zebrafish glypican genes are frequently enriched in adjacent cells. Because Glypicans can have different affects on cell signaling when expressed on opposing cell surfaces, our data suggests that the direct comparison of glypican function from zebrafish to higher vertebrates may be significantly limited.

343. Dynamic Diversity of Antigen Processing and Histocompatibility Genes within the Zebrafish Core MHC Class I Locus. ***Sean C. McConnell***[1], *Ross Kettleborough[2], Ian Sealy[2], Derek L. Stemple[2], Jill L.O. de Jong[1]*. 1) University of Chicago, Chicago, IL 60637; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK.
While the zebrafish is established as an animal model for stem cell biology, immunology and cancer research, histocompatibility is not well understood in this organism. We recently identified a set of ten distinct MHC class I genes linked to a core MHC locus on chromosome 19. Here we demonstrate genetic diversity among novel MHC class I haplotypes in zebrafish that is exceptional among vertebrates examined to date. Class I sequence comparisons show extremely high levels of sequence diversity, indicating that the different sequences are of ancient origin. Furthermore, phylogenetic examination of individual class I domains frequently shows higher similarity between zebrafish genes and genes from distantly related species (such as herring), than in comparisons with other zebrafish genes, providing evidence of trans-species polymorphism. These zebrafish class I sequences have several phylogenetically conserved substitutions within peptide anchor residues that are likely to influence peptide binding. We find evidence of recombination among the class I sequences, concentrated around the antigen presentation domains, which may shuffle these different sequences and influence their functional roles within the zebrafish class I pathway. We also examine two forms of psmb8 distributed among disparate haplotypes at this locus, with substitutions that are likely to affect antigen processing and class I presentation. Exome sequencing of a clonal fish line (CG2) expressing the divergent psmb8f allele suggests deletion of a large genomic region flanking the MHC core locus. This deleted region contains the abcb3 (tap2) gene and other genes involved in antigen processing. In conclusion, the haplotype variability that we describe is likely to increase antigen presentation diversity by isolating altogether different genes with their unique properties among the divergent haplotypes. These findings argue for a role for selection in maintaining alternative, divergent sequences within immune loci. Genomic sequencing will now help shed light on the mechanisms shaping this immunogenetic diversity.

344. Acute versus chronic alterations in metabolic state differentially effect hematopoietic stem cell function. ***Sung-Eun Lim***[1], *Mauricio Cortes[1], Wolfram Goessling[2,3], Trista E. North[1]*. 1) BIDMC; 2) BWH; 3) DFCI, Harvard Medical School, Boston MA 02115.
Metabolic disorders are a leading cause of morbidity and mortality, with gestational diabetes specifically impacting embryogenesis. The impact of elevated glucose levels on the developing hematopoietic system has not been resolved. Intriguingly, children born to diabetic mothers have a higher risk of developing childhood leukemia, suggesting increased blood sugar concentrations may have lasting impact on hematopoiesis. We recently showed glucose metabolism controls the onset and magnitude of HSC induction *in vivo*. In zebrafish, transient glucose elevation elicited dose-dependent effects on HSCs, including increased runx1 expression, as seen by *in situ* hybridization (ISH), qPCR, FACS, and hematopoietic cluster formation. This expansion was dependent on elevated metabolic activity and subsequent ROS-mediated induction of hif1a. In contrast to the relatively beneficial response of HSCs to moderate flux in metabolic rate, chronic glucose elevation during embryogenesis leads to lineage skewing during larval stages resulting in elevated erythrocyte and myeloid cells, with decreased lymphoid production, as seen by ISH, qPCR ($p<0.001$), and FACS ($p<0.05$). These results are due in part to hif1a-mediated transcriptional regulation, as the hif1 antagonist YC1 blocked the increase in myeloid cells. Ablation of islet cell-mediated insulin production via metronidazole exposure of transgenic *ins:nfsB-mCherry* embryos similarly antagonized myeloid lineage dysregulation. Further, phosphorylation of FOXOs1/3/4, downstream targets of insulin signaling, was increased in glucose treated embryos. Microarray analysis of *CD41:gfp* HSCs sorted from embryos exposed to chronic glucose elevations identified additional putative pathways contributing to the lineage bias. Investigations to determine if chronic metabolic dysregulation may contribute to progression to leukemia via these pathways are underway. Together, these studies indicate that both acute and chronic alterations in metabolic state affect HSC function and may further explain immunological phenotypes associated with metabolic syndrome and diabetes.

345. Inhibition of endothelial ERK signaling by Smad1/5 is essential for hematopoietic stem cell emergence. ***Feng Liu***. Institute of Zoology, CAS, Beijing, China.
The earliest hematopoietic stem cells (HSCs) are derived from hemogenic endothelium via endothelial-to-hematopoietic transition during vertebrate embryogenesis; however the underlying mechanism is largely unclear. Here we show that interplay of Smad1/5 and ERK

signaling is essential for hemogenic endothelium-based HSC emergence. Smad1/5 directly inhibits erk expression through recruiting HDAC1 to and inducing de-acetylation of the erk promoter in endothelial cells. Over-activated ERK signaling conferred by inhibition of Smad1/5 promotes the arterial endothelial cell fate and constitutively strengthens the tight junction between endothelial cells, thereby repressing the specification of hemogenic endothelium and the following endothelial-to-hematopoietic transition process. These findings provide new insights into the in vitro generation of transplantable HSCs for potential clinical applications.

346. Extracellular matrix remodeling by matrix metalloproteinases 2/9 is required for hematopoietic stem cell emergence and migration in the developing zebrafish embryo. *Mauricio Cortes[1], Kelsey Natsuhara[2], **Sarah Y Liu**[1], Wolfram Goessling[3], Trista E North[1].* 1) Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA; 2) Harvard University, Cambridge, MA; 3) Brigham and Women's Hospital/Harvard Medical School, Boston, MA.
The extracellular matrix (ECM) is responsible for modulating cell-cell interactions, cell migration and growth factor signaling. To elucidate the role of the ECM in hematopoietic stem cell (HSC) biology a screen of matrix metalloproteinase inhibitors identified MMP2/9 as modulators of HSCs in zebrafish embryos. MMP2 is broadly expressed from 12-36hpf with strong expression in the vascular niche. In contrast, MMP9 expression is restricted to a population of myeloid cells, with peak expression at 30hpf. Definitive HSCs are born from hemogenic endothelium in the aorta-gonad mesonephros (AGM) region, migrate and expand in the caudal hematopoietic tissue (CHT) and eventually colonize adult hematopoietic organs, the thymus and kidney marrow (KM). Based on their spatiotemporal expression, we hypothesized that MMP2/9 were required during definitive hematopoiesis. Treatment with ARP101 during HSC emergence (12-36hpf) resulted in accumulation of runx1+ cells in the AGM as well as of MMP2 substrates fibronectin and laminin in the niche. Consistent with that phenotype, MMP2/9 inhibition from 12-72hpf resulted in abnormal migration to the CHT. Similarly, MMP2 morpholino knockdown phenocopied the induction and migration defects observed by chemical inhibition. Embryos exposed to ARP101 from 42-96hpf, during thymus and KM colonization, showed reduced rag1+ cells in the thymus by WISH and FACS indicating reduced migration of lymphoid progenitors. In sum, our findings indicate that MMP2 is required for ECM remodeling in the hematopoietic niche, allowing for proper budding and migration of HSCs between sites of hematopoiesis. We had previously identified MMP9 as a downstream target of prostaglandinE2 (PGE2) on HSCs. Co-treatment with PGE2 and ARP101 resulted in the attenuation of the PGE2 enhancement of HSC production during embryonic development and in adult irradiation recovery assays, supporting our hypothesis that MMP9 is a component of the stimulatory effect of PGE2 on HSCs.

347. Pdgfr signaling is required for specification of hematopoietic stem cells. ***Chase H. Melick**, Albert D. Kim, Josh Bloomekatz, David Traver.* University of California San Diego, La Jolla, CA. 92093.
Hematopoietic stem cells (HSCs) are responsible for the replenishment of all adult blood cells throughout the lifespan of an individual. For this reason they are extremely useful in clinic for the treatment of a multitude of hematological abnormalities such as leukemia or lymphoma, however the need for immune matching between patient and donor bone marrow is a major limitation. Thus a current research focus for regenerative medicine and stem cell biology is to establish transplantable hematopoietic stem cells from embryonic stem cells or induced pluripotent cells (iPSCs) in order to treat patients, however this feat has not been achieved and indicates that our understanding for the molecular mechanisms involved in HSC generation are incomplete. Many signaling pathways are known to be critical for the successful generation of HSCs in vivo, however a role for Pdgfr signaling better known for a role in vascular tissues, has not been investigated. We treated embryos at different developmental temporal windows with a pharmacological inhibitor for tyrosine kinase activity of Pdgfr. Surprisingly we found that Pdgfr signaling is required for HSCs well before the formation of HSCs during early somitogenesis, later Pdgfr signaling is required for intersegmental vessel sprouting but is dispensable for HSC formation as previously reported. The loss of HSCs during this early drug treatment window accompanied by defects to dorsal and somitic tissues, suggesting that these phenotypes may be related. Formation of dorsal aorta, sclerotome, and HSCs is a Notch-signaling dependent process, we therefore asked if Pdgfr signaling was related to Notch signaling. We found that activation of Notch signaling in the endothelium was inhibited in the context of Pdgfr inhibition, furthermore we found that ectopic activation of Notch signaling rescued HSCs in these embryos suggesting a novel pathway by which Pdgfr signaling is involved in HSC specification.

348. Autocrine and paracrine TGFb signalling is required for the emergence of haematopoietic stem cells. ***Rui Monteiro**[1,2], Philip Pinheiro[1], Tessa Peterkin[1], Roger Patient[1,2].* 1) Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; 2) BHF Centre of Research Excellence, Oxford, United Kingdom.
Haematopoietic stem cells (HSCs) are self-renewing multipotent progenitors which generate and maintain all mature blood cells. They emerge from arterial endothelial cells in the floor of the dorsal aorta which are known collectively as the hemogenic endothelium. Programming endothelial cells to become HSCs is therefore critical to inform on attempts to generate these cells in vitro. By knocking down the Transforming Growth Factorb receptor 2 (TGFbR2), we demonstrate for the first time that TGFb signalling is critically required for HSC emergence. The requirement for TGFb signalling is two-fold: paracrine, via *tgfb3* expressed in the neighbouring notochord, and autocrine, via *tgfb1a* and *tgfb1b*, expressed in the arterial endothelium that gives rise to the HSCs. Epistatic analyses revealed that TGFb signalling is independent of Notch and that Vegf signalling regulates *tgfb1a* and *tgfb1b* expression. We further show that TGFb regulates cell cycle progression in the hemogenic endothelium via indirect repression of *p53* and *cdkn1a* by the key transcription factor Runx1. In summary, our findings provide evidence for a novel role for paracrine and autocrine TGFb signalling in the generation of HSCs in vertebrates.

349. Dissecting the role of the ETV6 during zebrafish development. ***Parisa Rasighaemi***[1], *Sara Onnebo*[2], *Clifford Liongue*[1], *Alister C.Ward*[1]. 1) School of Medicine, and Strategic Research Centre in Molecular and Medical Research, Deakin University, Geelong, Victoria, Australia; 2) School of Life & Environmental Sciences, Deakin University, Burwood, Victoria, Australia.
Chromosomal translocations involving fusions of the human ETV6 gene occur frequently in hematological malignancies. Interestingly, in cells harbouring ETV6 translocations the second ETV6 allele is often mutated as well, suggesting a role for ETV6 as a negative regulator within the haematopoietic transcriptional hierarchy. However, a detailed understanding of the normal function of ETV6 remains incomplete. This study has employed zebrafish as a relevant model to investigate the role of ETV6 during embryonic hematopoiesis. Bioinformatics analysis revealed that zebrafish possessed a single conserved ETV6 orthologue. The spatio-temporal expression profile of the zebrafish ETV6 using WISH revealed that ETV6 transcripts were first evident at 12 hpf in the lateral plate mesoderm, which contains precursors for both blood and vasculature. By 24 hpf ETV6 expression was observed in the posterior blood island, the site of erythro-myeloid progenitor production during the primitive wave of haematopoiesis. By 6 dpf ETV6 expression was also seen in thymus and mesonephric duct which are the site of T-lymphocyte and blood cell production during definitive hematopoiesis respectively. The possible involvement of zebrafish ETV6 during hematopoiesis was investigated by morpholino-mediated gene knockdown and subsequent analysis of hematopoietic and immune cells using a variety of molecular techniques. During primitive hematopoiesis, ETV6 knockdown resulted in reduced levels of progenitor cells, erythrocyte and macrophage populations, but increased numbers of incompletely differentiated heterophils. During definitive hematopoiesis, ETV6 knockdown decreased HSCs, erythrocytes and myeloid cells, but resulted in enhanced lymphopoiesis. This suggests that ETV6 plays a broader and more complex role in hematopoiesis than previously thought.

350. CaMK-II Regulates HSC Specification. ***Sarah Rothschild****, Wilson Clements.* Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN.
Hematopoietic stem cells (HSCs) are a self-renewing population of precursors that can proliferate and differentiate into all blood and immune cells over the lifetime of an individual, and comprise the therapeutic component of bone marrow transplants. Understanding the developmental specification of HSCs is a key clinical goal to inform in vitro directed differentiation of pluripotent cells. We have identified a previously unknown regulator of HSC specification, the multifunctional Ca^{2+}/calmodulin-dependent protein kinase type 2 (CaMK-II). In zebrafish, pharmacological inhibition of CaMK-II or loss of function in at least one specific gene, camk2g1, prevents specification of the earliest HSCs. Interestingly, expression of bmp4, which is required for HSC specification, is also locally abrogated in camk2g1 knock down animals. These data represent the first known instance of CaMK-II regulation of Bmp signaling. Our results suggest that CaMK-II may direct embryonic HSC specification is through regulation of Bmp signaling.

351. Zebrafish Archive Manager (ZAM): A web application to store and monitor zebrafish husbandry information. *Richard C. Dawson, Jody Rosenblatt*, ***George T. Eisenhoffer***. Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.
The zebrafish Danio rerio has become an attractive model system to study the genetics of development and disease. Zebrafish are small, easy to culture, and the wide array of existing transgenic and mutant lines provide a tremendous resource for biomedical research. Maintenance of husbandry records is both time intensive and laborious, and a standardized way to manage and track the large amount of zebrafish lines in a given laboratory or centralized facility has been lacking. Here we describe a web-based application entitled Zebrafish Archive Manager (ZAM) that provides a rapid, secure and easy way to store and monitor information associated with zebrafish aquaculture. ZAM provides a standardized system to record and track individual strains of zebrafish from generation to generation for both individual laboratories and large centralized facilities. ZAM is an open-source flexible platform that can import existing husbandry data from a variety of formats, control access of the stored information to different users, track individual tanks using a QR/barcode system, and send automated email updates concerning zebrafish age and statistics. Importantly, the ZAM application is compatible with common smart phones to set crosses and track pedigree information, saving time and effort from manually entering the data on a computer. ZAM also includes separate searchable databases for plasmid stocks used to generate transgenic lines and for lines that have sperm frozen for long-term preservation of the genetic background. In sum, ZAM is a freely available web application that provides a uniform format for the tracking and storage of zebrafish husbandry information.

352. Husbandry Guidelines for New Labs and Expanding Research Protocols. ***Chris Obenschain****, Zebrafish Husbandry Association.* Tecniplast, 1345 Enterprise Drive, West Chester, PA.
This session will focus on the needs of individuals and groups starting new facilities and those expanding their use of Zebrafish as a research model. As investigators begin or expand their use of Zebrafish as a research model, the topics discussed will give those individuals and groups a basis for design and standard operating procedure development. Utilizing the Zebrafish Husbandry Association members the information provided will be timely and pertinent to todays Zebrafish laboratory needs.

353. Regulation of macrophage inflammation in a larval zebrafish model of *Streptococcus iniae* infection. ***Elizabeth A. Harvie***[1], *Anna Huttenlocher*[1,2]. 1) Department of Medical Microbiology and Immunology, University of Wisconsin - Madison, Madison, WI; 2) Department of Pediatrics, University of Wisconsin - Madison, Madison, WI.
The optical translucency and genetic tractability of the larval zebrafish make it an attractive model for the real-time in vivo analysis of host-microbe interactions. Our previous work has shown that both neutrophils and macrophages are important for the control of the fish and human pathogen, *Streptococcus iniae*. Here, we report that as part of the host inflammatory response to infection, macrophages form organized aggregate structures, and this macrophage inflammatory response seems to be mediated, at least in part, by leukotriene B_4 (LTB_4) production. Live confocal imaging of transgenic fluorescently-labeled zebrafish larvae demonstrated that both neutrophils and macrophages were recruited to localized otic infection with *S. iniae*. In addition to the localized inflammatory response, we also observed organized macrophage inflammation in the tail of infected fish. Proper neutrophil function was found to be necessary for this macrophage

aggregation because the same macrophage response did not occur in a transgenic zebrafish with impaired neutrophil function. This led to the hypothesis that neutrophils may be releasing factors that control the macrophage response to infection. LTB_4 is a potent leukocyte chemoattractant and pro-inflammatory eicosanoid produced by immune cells. Morpholino-mediated knock down of leukotriene A_4 hydrolase (LTA4H), which catalyzes the last step in LTB_4 synthesis, resulted in the abrogation of macrophage inflammation. Treatment of LTA4H deficient fish with exogenous LTB_4 rescued this inflammatory defect. Taken together, we have found that neutrophils regulate macrophage inflammation in response to *S. iniae* infection in zebrafish larvae, possibly through LTB_4.

354. Distinct innate immune responses to *Aspergillus fumigatus* conidia and hyphae in zebrafish larvae. ***Benjamin Knox***[1,2], *Nancy Keller*[2,3], *Anna Huttenlocher*[2,4]. 1) Microbiology Doctoral Training Program, University of Wisconsin-Madison, Madison, Wisconsin; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin; 3) Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin; 4) Department of Pediatrics, University of Wisconsin-Madison, Madison, Wisconsin.
Aspergillus fumigatus is the most common filamentous fungal pathogen of immunocompromised individuals resulting in invasive aspergillosis (IA) and high mortality rates. While innate immunity is known to be the predominant host defense against *A. fumigatus*, innate phagocyte responses to *A. fumigatus* and their contributions to host survival in an intact host remain unclear. Here, we describe a larval zebrafish *A. fumigatus* infection model amenable to real time imaging of host-fungal interactions in live animals. Following infection, innate phagocyte populations exhibit clear preferences for different fungal morphologies: macrophages rapidly phagocytose conidia and form aggregates around hyphae while the neutrophil response is dependent upon the presence of hyphae. Depletion of macrophages rendered host larvae susceptible to invasive disease. Moreover, a zebrafish model of human leukocyte adhesion deficiency with impaired neutrophil function also resulted in invasive disease and diminished host survival. In contrast, macrophage-deficient but not neutrophil-deficient larvae exhibited attenuated disease following challenge with a less virulent strain of *A. fumigatus*, *DlaeA*, which has defects in secondary metabolite production. Taken together, we have established a new and accessible vertebrate model for studying innate immune responses to *A. fumigatus* in an intact host that reveals distinct roles for neutrophils and macrophages in mediating host defense against IA.

355. Lysosomal Homeostasis Regulated by Spns1 and Spns3 in Zebrafish. *Shanshan Lian*[1], *Tomoyuki Sasaki*[1], *Wenbiao Chen*[2], ***Shuji Kishi***[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Vanderbilt University School of Medicine, Nashville, TN.
Autophagy is a cellular stress response upon various nutrient conditions, and it involves a complex catabolic program for lysosomal degradation of proteins as well as other subcellular constituents. Many of the metabolic wastes can also lead to the induction of oxidative stress and damage at the cellular level in various tissues. This can, subsequently, perturb organismal homeostasis leading to deteriorative conditions during the development and aging process as well as multiple disorders such as neurodegeneration, liver disease, muscle and heart diseases and cancer. However, the mechanism of deteriorations in intrinsic catabolism and stress signaling pathways is a complex enigma and still remains obscure. Particularly, it remains unknown how the late stages of the autophagy are coupled to signaling machinery for lysosomal degradation in response to stress. Spinster (Spin) in *Drosophila* or Spinster homolog 1 (Spns1), one of the three different homologs (Spns1-3) in vertebrates, is a hypothetical lysosomal H^+-carbohydrate transporter in the major facilitator superfamily (MFS) related to the arabinose efflux permease. Spns1 functions at a late stage of autophagy, and loss of Spns1 leads to the accumulation of enlarged autolysosomes that presumably fail to degrade their contents and/or to effluent degraded contents. Still little is known, however, about the molecular mechanism leading to pathogenesis accompanied with such enlarged autolysosomes at organismal levels in vivo. Spns2 has been well documented about its function as a sphingosine 1-phosphate (S1P) transporter localized at the plasma membrane, which plays important roles in diverse cellular functions such as cell proliferation, differentiation and migration. However, very little is known about the functional role(s) of Spns3 that colocalizes with Spns1 at the lysosomal membrane. It is, therefore, our intention to identify the genetic pathway linked to vertebrate Spns1 and 3, as compared with *Drosophila* Spin. We also aim to find chemical and/or genetic modulators of autophagic and stress responses and study their effects on lysosomal homeostasis and catabolic traits at the organismal level using zebrafish as an amenable vertebrate model system.

356. Hyperinsulinemia induces insulin resistance and immune suppression via Ptpn6/Shp1 in zebrafish. ***Rubén Marín-Juez***[1], *Herman P. Spaink*[2]. 1) ZF-Screens BV, Leiden, Netherlands; 2) Institute of Biology, Leiden University, Leiden, Netherlands.
Type 2 Diabetes, obesity and metabolic syndrome are pathologies impacting a large population worldwide and where insulin resistance plays a central role. These pathologies are usually associated with a dysregulation of insulin secretion leading to a chronic exposure of the tissues to high insulin levels (i.e. hyperinsulinemia), which diminishes the concentration of key downstream elements causing insulin resistance. The complexity of the study of insulin resistance arises from the heterogeneity of the metabolic states where it is observed. In consequence, animal models for the study of insulin resistance, can not completely recapitulate the metabolic status of insulin resistant humans, which is translated in contradictory observations. To contribute to the understanding of the mechanisms triggering insulin resistance we have developed a zebrafish model to study insulin metabolism and its associated disorders. Zebrafish larvae appeared to be sensitive to human recombinant insulin, becoming insulin resistant when exposed to a high dose of the hormone, as confirmed by glucose measurements. Moreover RNAseq-based transcriptomic profiling of these larvae revealed a strong down regulation of a number of immune relevant genes as a consequence of the exposure to hyperinsulinemia. Interestingly, as an exception, the negative immune modulator *ptpn6* appeared to be up regulated in insulin resistant larvae. Knockdown of *ptpn6* showed to counteract the observed down regulation of the immune system and insulin signaling pathways effects at the transcriptional level caused by hyperinsulinemia. These results show that *ptpn6* is a mediator of the metabolic switch between insulin sensitive and insulin resistant states. Our zebrafish model for hyperinsulinemia has therefore demonstrated its suitability to discover novel regulators of insulin resistance. In addition, our data will be very useful to further study the function of immunological determinants in a non-obese model system.

357. Using Zebrafish to Model Human Developmental Disorders Linked to Folic Acid Through Pharmacologic and Genetic Perturbations of Folate Metabolism. ***James T. Warren****, Samantha Storti, Paolo Londono.* Biology, Penn State Erie, Erie, PA., 16563.
Folic acid is a B-vitamin that has been linked to numerous developmental and adult onset disorders in humans. This vitamin is a cofactor in dozens of biochemical reactions that involve single carbon transfers, including the metabolism of amino acids, nucleic acids, neurotransmitters and methylation reactions. Folic acid was first associated with the development of neural tube defects (NTDs) over fifty years ago, and it has been shown that a periconceptional administration of folic acid can prevent up to 70% of NTDs. In recent years, folic acid has been implicated in the etiology of numerous other developmental and adult onset disorders, such as heart septation defects, cancer, high blood pressure, irritable bowel syndrome, psychological disorders, Alzheimer's Disease and arteriosclerosis, to name a few. Despite the long-standing and wide interest in the involvement of folic acid in these disorders, there has been limited progress in elucidating precisely which of the dozens of biochemical reactions linked to folate metabolism are the major contributors to disease. The zebrafish is an excellent model system to address this problem. Our laboratory has pharmacologically perturbed folate metabolism by exposing developing zebrafish embryos to homocysteine, a metabolite seen in elevated levels in many of the above mentioned folate linked disorders. These embryos display a variety of developmental defects, including neural tube defects, heart formation and circulation abnormalities and pigmentation defects. We are now using morpholino oligonucleotides to genetically perturb folate metabolism and systematically knock down the key enzymes using this vitamin. This study will summarize the phenotypes that result from the knockdown of the genes for two key enzymes that lead to the breakdown of homocysteine; methionine synthase and betaine homocysteine methyl transferase.

358. Scarb2a is essential for Notochord Development in Zebrafish. ***A. Diaz Tellez****, C. Zampedri, J. Ramos Balderas, S. Carrillo Rosas, E. Maldonado*. Biologia del Desarrollo Dept, IFC, UNAM.
Scarb2a is a membrane glycoprotein, whit two tran-membrane sites, 11 sites for N-glycosylation and a C-terminal di-leucine motif. Mutations in Scarb2 were described as causing of Action Myclonus Renal Failure Syndrome (AMRF), which is characterized by progressive myoclonus epilepsy. Zebrafish has three copies of scarb2 (scarb2a, scarb2b and scarb2c). Scarb2a insertional-mutant was obtained in a large-scale forward genetic screening. This mutant is characterized by the presence of vesicular bodies in the brain at 1 dpf and hypopigmentation at 2 dpf both phenotypes are restored at 3 dpf. However, since 1 dpf scarb2a mutant shows defects in the notochord formation. Through electronic microscopy, we have observed that vacuole notochord cells of scarb2a mutant are smaller. In situ hybridization revealed that scarb2a is expressed in the brain and in the notochord at early stages, also in the Scarb2a mutants there is a disordes in collagen expression. Actually , experiments are ongoing to decipher if the defect in the notochord of scarb2a Zebrafish mutant are cell autonomous or non-autonomous.

359. Exploring Mechanisms Underlying Craniofacial Malformations Resulting From Developmental Exposure To Benzo[A]Pyrene. ***J. M. Dickson***[1]*, D. R. Hammond-Weinberger*[2]*, A. J. Udvadia*[1]. 1) University of Wisconsin-Milwaukee, Department of Biological Sciences, Milwaukee, WI USA; 2) University of California-San Diego, Department of Biological Sciences, San Diego, CA USA (current address).
Early exposure to environmental toxins can have drastic effects on larval fish development. Benzo(a)pyrene, BaP, is an environmental toxin that has been reported to cause craniofacial defects in the rockfish, Sebasticus marmoratus. A search of the Comparative Toxicogenomic Database suggests that BaP impacts expression of genes encoding the histone H3 variant, H3.3, and the HIRA histone H3.3 chaperone complex, which is important in regulating the insertion of the histone variant H3.3 at actively transcribing genes. Dominant negative mutations in the H3.3a gene have recently been shown to cause gross craniofacial defects (Cox et al. 2012, PLOS Genetics). Furthermore, our own preliminary data show that knockdown of CABIN1, a member of the HIRA complex, also results in craniofacial defects. Some of the major craniofacial defects that we found in CABIN1 knockdown fish were in the ventral cartilages that will eventually form the lower jaw and support the gills, including the Meckel's cartilage, ceratohyoid cartilage, and cartilages of the posterior branchial arches. Defects in these structures have also been reported in rockfish embryos that were exposed to BaP. Therefore we hypothesize that early environmental exposure to BaP causes craniofacial defects by disrupting H3.3 incorporation during development of the cranial neural crest. We are currently quantifying effects of environmentally relevant levels of BaP on ventral cartilage formation in developmentally exposed zebrafish. We are also quantifying the differences in the expression patterns of key genes involved in chondrogenesis in zebrafish developmentally exposed to BaP. This study is an important step in understanding how early exposure to BaP can lead to changes in epigenetic regulation that will affect craniofacial development.

360. *Mau*/Aqp3a is a novel regulator of zebrafish pattern formation and fin growth. ***Anastasia Eskova***[1]*, Uwe Irion*[1]*, Jana Krauss*[1]*, François Chauvigne*[2]*, Moritz Ammelburg*[1]*, Joan Cerdà*[2]*, Christiane Nüsslein-Volhard*[1]. 1) MPI for Developmental Biology, Tübingen, Germany; 2) IRTA-ICM, Barcelona, Spain.
The skin pattern of zebrafish is formed by three types of chromatophores: black melanophores, yellow xanthophores, and iridescent iridophores. To create the characteristic horizontal stripes, chomatophores interact with each other and the surrounding tissues. Although a number of regulators of the stripe formation, pigmentation, and pigment cell development have been described, the underlying mechanisms of these processes remain elusive and controversial.
Here we present the water and glycerol channel Aquaporin 3a as a novel regulator of stripe formation in adult zebrafish. Chemical mutagenesis screens identified four dominant mutations of *mau* gene, coding for Aqp3a. All of them carry amino acid substitutions in conserved positions which are, according to bioinformatic prediction, at the pore surface of aquaporin. All four mutations lead to a similar phenotype: although all the chromatophore types are present in the skin, the mutants display breakages in stripes. In addition, they have short fins, with fewer normally sized fin ray segments. Both phenotypes are not present in larvae, but appear during metamorphosis. Experiments in *Xenopus* oocytes show that all four mutant alleles of *mau* are deficient in water and glycerol transport. Interestingly, three of them partially or completely retained in the ER, when expressed in mammalian cells and overexpressed in zebrafish embryos. Cell

transplantations indicate that mutations in *mau* interfere with melanophore behaviour rather than with functioning of xanthophores or iridophores. However, given the strong expression of of *mau* in the epidermis, it is suggested, that the tissue environment is one of the main players in the chromatophore organization. Several gap junction and ion channel proteins have been associated with the patterning of zebrafish skin and regulation of fin size. This is the first report of the involvement of water and glycerol channels in these processes, which offers a new dimension to our view on interactions regulating chromatophore patterning, and fin development.

361. Identifying Putative *Vangl2* Dependent Genes in the Developing Zebrafish Hindbrain - Transcriptomic and Proteomic Analyses. *V. Sittaramane[1,2], X. Pan[1], **S. Gurung**[1], P. Singh[1], A. Chandrasekhar[1].* 1) University of Missouri, Columbia, MO; 2) Georgia Southern University, Statesboro, GA.
The membrane protein Vangl2, a core component of Wnt/Planar Cell Polarity (PCP) pathway, functions independently of PCP signaling to regulate the caudal migration of facial branchiomotor (FBM) neurons in the vertebrate hindbrain. We showed previously that Vangl2 genetically interacts with the cell adhesion molecule Tag1 and extracellular matrix (ECM) protein Laminina1 during migration of FBM neurons in zebrafish. However, the mechanisms underlying these genetic interactions are obscure. In an attempt to shed light on these mechanisms, we sought to identify genes whose expression might be altered by *vangl2* knockdown. Therefore, we performed proteomic (2D-DIGE) and transcriptomic (RNA Seq) analyses using zebrafish hindbrain explants from control and *vangl2* morphant embryos. Proteomic analysis identified putative candidates, including Hsp90b, which has recently been shown to have a non-mitochondrial signaling role at the cell surface. The RNA Seq analysis identified several candidates, including transmembrane ligands Sema6dl and Lrrn1. Importantly, the analyses also identified a vesicular transport protein Sec24b and laminin-binding integrin Itga6 that have demonstrated roles in mediating Vangl2 function, and in FBM neuron migration, respectively, validating the expression profiling data. Lrrtm1, a transmembrane receptor that binds Lrrn1 and plays a role in synaptogenesis, is expressed in FBM neurons. Sema6dl, which has several putative co-receptors, including integrins, is expressed in specific hindbrain rhombomeres during the period of FBM neuron migration. These data suggest that we have identified signaling molecules that potentially function together with Vangl2 to regulate neuronal migration. We are currently generating loss of function mutations in these genes using CRISPR-Cas to directly examine their roles in FBM neuron migration.

362. Molecular mechanisms of somal translocation of ganglion cells during zebrafish retinogenesis. ***J. Icha**, R. Swane, C. Norden.* MPI-CBG, Dresden, Germany.
The zebrafish retina is an outstanding model to investigate morphogenesis of the central nervous system. We use it to study the migration of newly born neurons undergoing somal translocation. Cell divisions early in retinogenesis are confined to its apical side. We investigate migration of the neurons born first, the ganglion cells (RGCs). They are destined to form the basal retinal layer and therefore have to migrate basally to their final destination. We found that they do so by translocating their soma over a distance of 50 mm in around three hours. Despite the widespread occurrence of somal translocation in the central nervous system and importance of correct positioning of ganglion cells for vision, the molecular mechanisms underlying somal translocation remain unknown. We set out to investigate the role of different organelles and the cytoskeletal components during RGC translocation using timelapse lightsheet and confocal microscopy. Looking at the localization of different organelles during the translocation, we found that both the centrosome and Golgi apparatus remain in the apical tip of the cell, which is rearward from the nucleus, arguing against active involvement of these organelles. Actin and myosin were evenly distributed in migrating RGCs so we concluded that a direct role of this machinery is unlikely. In contrast, we saw a specific accumulation of stabilized microtubules and dynein in the apical process of migrating cells. Microtubule plus tip tracking revealed antiparallel arrangement of filaments in this apical microtubule bundle. When we used drugs to inhibit dynein or depolymerize microtubules, RGC translocation was suspended. This showed that the stable microtubules and dynein in the apical process are required for RGC somal translocation. The next steps will be to test whether the microtubules rearward from the nucleus are the force generating entity, or whether their function is rather passive as a barrier for the soma to move back apically. In the long term we aim to describe the force generating mechanism of RGC somal translocation in detail. We expect to gain new insight into mechanisms of somal translocation that might also come into play in other parts of the nervous system.

363. Understanding planar cell polarity in migrating cells. ***Jason R. Jessen**[1], Michael Dohn[2], Julia Buckley[1], Tammy Jessen[1].* 1) Middle Tennessee State University, Murfreesboro, TN; 2) Vanderbilt University Medical Center, Nashville, TN.
My laboratory aims to understand how planar cell polarity (PCP) proteins regulate cell migration and morphogenetic events underlying embryonic development. During zebrafish gastrulation, loss of PCP gene function disrupts polarity as indicated by changes in cell elongation, orientation, and membrane protrusive activity. Zebrafish mutants or morphants of PCP proteins such as Vang-like 2 (Vangl2), Prickle1a, Glypican4, and Frizzled7 exhibit gastrulation defects characterized by shortened and broadened body axes. Our research has identified functional relationships between Vangl2 and membrane type-1 matrix metalloproteinase (Mmp14) activity and between Glypican4 and cadherin-mediated cell adhesion. While the mechanisms for these interactions are unclear, our accumulating data demonstrate that PCP proteins differentially regulate signaling pathways that impact ECM organization during zebrafish gastrulation. After recently moving my laboratory to a new institution, we initiated three experimental approaches aimed at uncovering mechanisms regulating PCP in migrating cells. First, we have conducted a proteomics screen to identify Vangl2 binding proteins that might facilitate Vangl2-dependent regulation of cell surface Mmp14 proteolytic activity and ECM degradation. Confirmation and analyses of the top hit(s) will be presented. Second, we are utilizing TALENS to generate Mmp14 mutant zebrafish lines with the goal of more clearly defining the role of this protease during gastrulation. We have thus far identified founder zebrafish with premature stop codons and mutations in the Mmp14 catalytic domain and are currently raising F1 generations. Phenotype analyses will be presented. Lastly, having demonstrated that *knypek/glypican4* mutant embryos (*kny^{m119}*) have increased cell surface cadherin expression and increased cell-cell adhesion, we now hope to determine the relationship between this PCP protein and cadherin function. We are particularly interested in p120-catenin (CTNND1)

and reported connections between PCP proteins and p120-catenin regulation of cadherin function. Current efforts are focused on the analysis of gastrula cell membrane protrusion dynamics in situations of increased and decreased adhesion.

364. Mmp17b is essential for proper neural crest cell migration in vivo. ***Noah R. Leigh***[1], *Keguo Li*[1], *Marcus-Oliver Schupp*[1], *Padmanabhan Vakeel*[1], *Adam Gastonguay*[1], *Ling Wang*[1], *Chang Z. Chun*[3], *George A. Wilkinson*[2], *Ramani Ramchandran*[1]. 1) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Pharmacy, Concordia University, Mequon, WI; 3) Medicine, University of Florida, Gainesville, FL.
The extracellular matrix plays a critical role in neural crest (NC) cell migration. In this study, we characterize the contribution of the novel GPI-linked matrix metalloproteinase (MMP) zebrafish mmp17b. Mmp17b is expressed post-gastrulation in the developing NC. Morpholino inactivation of mmp17b function, or chemical inhibition of MMP activity results in aberrant NC cell migration with minimal change in NC proliferation or apoptosis. Intriguingly, a GPI anchored protein with metalloproteinase inhibitor properties, Reversion-inducing-Cysteine-rich protein with Kazal motifs (RECK), which has previously been implicated in NC development, is expressed in close apposition to NC cells expressing mmp17b, raising the possibility that these two gene products interact. Consistent with this possibility, embryos silenced for mmp17b show defective development of the dorsal root ganglia (DRG), a crest-derived structure affected in RECK mutant fish sensory deprived (sdp). Taken together, this study has identified the first pair of MMP, and their putative MMP inhibitor RECK that functions together in NC cell migration.

365. Early Craniofacial Defects Occur Following Knockdown of the Extracellular Matrix Protein, TINAGL1. *Hannah Neiswender*[1], *Sammy Navarre*[2], *David J. Kozlowski*[1,2], ***Ellen K. LeMosy***[1]. 1) Dept Cell Biol & Anatomy, Georgia Regents University, Augusta, GA; 2) Institute of Molecular Medicine and Genetics, Georgia Regents University, Augusta, GA.
The TINAGL family of secreted basement membrane proteins (TINAGL1 and TINAG in mammals; TINAGL1 in lower species) is highly conserved but functionally opaque. Limited data from humans and mice has suggested functions in cell adhesion, renal and vascular development, cranial suture closure, and suppression of metastasis, while the fly TINAGL1 appears to act as a positive Wnt (Wg) cofactor. We are using morpholino (MO) knockdown to study TINAGL1 function in zebrafish development. A consistent pattern of pharyngeal arch cartilage defects is observed with three independent TINAGL1 MOs but not their mis-match controls, and is observed with co-injection of sub-threshold doses of the two best-characterized MOs. Substantial rescue (e.g., 35% vs 95% larvae affected) is observed by co-injection of *tinagl1* mRNA. In situ hybridization demonstrates endogenous *tinagl1* expression in ventral tissues underlying the hindbrain at 15-22 hpf. In 24 hpf morphant zebrafish, the neural crest cell marker *dlx2a* is reduced or absent in posterior pharyngeal arch domains that show severe defects. These results suggest that TINAGL1 is required for survival or migration of neural crest cells in some domains of the pharyngeal apparatus. We postulate roles involving cell adhesion in tissues through which the neural crest cells migrate, or involving regulation of Wnt localization and activity within these tissues. Future experiments will address neural crest cell behavior in these morphants, and whether TINAGL1 genetically and/or physically interacts with Wnts important during early craniofacial development.

366. The Spadetail/Tbx16 Transcription Factor Regulates Zebrafish Mesodermal Cell Polarity. ***Alyssa J. Manning***, *David Kimelman.* Department of Biochemistry, University of Washington, Seattle, WA.
During early vertebrate embryonic development tissues become organized progressively from anterior to posterior along the body axis. In zebrafish, mesodermal precursor cells reside in the posterior end of the body, the tail bud, and gradually move out and differentiate to form somites. During this process they undergo a pseudo-epithelial to mesenchymal transition (EMT) and later undergo the reverse process, a mesenchymal to epithelial transition. Many of the precise movements of cells as they move from mesodermal progenitor through a maturation stage to presomitic mesoderm and finally to somites have not been described in detail. The T-box transcription factor Spadetail/Tbx16 (Spt) is known to be required both for mesodermal cell morphogenesis and differentiation. Previous data suggest that *spt* mutant mesodermal progenitor cells can initiate but not complete EMT, leaving the pseudo-epithelium but not acquiring all of the correct migratory properties, and therefore accumulate at the posterior end of the embryo. Spt targets required for cell morphogenesis are not known. I have developed a tail bud explant method to monitor the migration of cells and their actin cytoskeleton in the maturing mesoderm. Fixed and live imaging of tail bud explants during somitogenesis revealed that the numbers and types of actin-based protrusions are the same in wild-type, *spt* morphant and Spt overexpressing cells. However, premesodermal cells with altered Spt levels cannot orient their cell protrusions properly. These data suggest that Spt is required in mesodermal cells for acquisition of migratory polarity during maturation, though not for protrusion formation. Examination of larger scale cell movements in wild-type, *spt* mutants and morphants, and Spt overexpressing animals are ongoing. Additionally, RNA-Seq is being used to discover Spt targets that may be required for cell polarity and migration orientation.

367. A dual role for *atp1b1a* in osmoregulation and epidermal homeostasis. ***Julia Hatzold***, *Filippo Bellegia, Hannah Herzig, Wilhelm Bloch, Bernd Wollnik, Matthias Hammerschmidt.* University of Cologne, Germany.
The epidermis serves as a barrier to protect the organism not only from mechanical stressors, but also from physiological challenges such as different environmental pH and salt concentrations. Zebrafish *psoriasis* mutants display epidermal aggregates, resulting from disrupted keratinocyte proliferation and differentiation (Webb, Driever, Kimelman, Dev Dyn 2008). We conducted whole exome sequencing of *psoriasis* mutants and identified a causative mutation in Atp1b1a, the b subunit of a Na-K-ATPase ion pump composed of a catalytic a- and a regulatory b-subunit. The pump transports Na+ and K+ across the cell membrane, thereby playing a well-characterized role in generating an electrochemical gradient. However, multiple pump-dependent and -independent functions in signaling and cell adhesion have been described as well. Zebrafish *atp1b1a* is weakly expressed in the skin and more strongly in the pronephric ducts (PD). Accordingly, *psorasis* mutants display defects pointing to compromised renal osmoregulation, such as a loss of the basolateral localization of the a subunit in PD cells, dilated PDs and the development of edemas. Inhibition of pump activity by ouabain in wt embryos phenocopies the edema formation

of *psoriasis* mutants, but fails to induce epidermal aggregates, pointing to a PD- and pump-independent role of *atp1b1a* in the epidermis. TEM studies and permeability assays reveal mild alterations in epidermal cell junctions and impaired barrier function. Surprisingly, epidermal aggregate formation is rescued upon incubation of *psoriasis* mutants in isotonic medium, indicating that the epidermal function of *atp1b1a* is only required in hypotonic conditions. The data are in line with previously reported effects of hypotony on keratinocyte differentiation / proliferation *in vitro* and point to a crucial impact of osmotic stress on epidermal homeostasis *in vivo*. In conclusion, we propose that the b subunit of the Na-K-ATPase is required in the skin to enforce cell adhesion and to sustain the osmotic pressure caused by the hypotonic environment. Moreover, it is also directly involved in water balance regulation by strengthening the epidermal barrier to restrict water influx and promoting PD-dependent water efflux.

368. Chondroitin / Dermatan sulfate modifying enzymes in zebrafish development. *Judith Habicher[1], Tatjana Haitina[1], Inger Eriksson[2], Katarina Holmborn[1], Tabea Dierker[2], Per E. Ahlberg[1], **Johan Ledin**[1]*. 1) Department of Organismal Biology, Science of Life Laboratory, Uppsala University, Uppsala, Sweden; 2) Department of Medical Biochemistry and Microbiology, Science of Life Laboratory, Uppsala University, Uppsala, Sweden.
Chondroitin/dermatan sulfate (CS/DS) are long unbranched sulfated glycosaminoglycans (GAGs), attached to serine residues on core proteins. The GAG biosynthesis is a complex process, where different enzymes catalyze modifications such as sulfation and epimerization in specific positions. Many extracellular matrix structural molecules and signalling factors bind to the CS/DS pattern of sulfation which is distinct in different tissues indicating regulatory roles in tissue development and homeostasis. Here we report a phylogenetic analysis and the early expression of zebrafish CS/DS modifying enzymes; sulfotransferases (*chst3a, chst3b, chst7, chst11, chst12a, chst12b, chst13, chst14, chst15, ust*) and epimerases (*dse, dsela and dselb*). In addition we present a biochemical characterization of CS/DS biosynthesis showing that CS/DS sulfation increases during larval development where 4-O-sulfation dominates and 6-O-sulfation increases in later developmental stages. Di-sulfated and 2-O sulfated disaccharides, synthesized by *chst15* and *ust* respectively, are rare and these enzymes display a restricted expression pattern. Interestingly, while expression of CS/DS glycosyltransferases as well as CS/DS deposition are concentrated to pharyngeal arches and the notochord (Filipek-Gorniok et al, 2013), only *chst7, chst11, chst12a* and *chst14* recapitulate this spatial restriction. This indicates that a majority of CS/DS modifying enzymes synthesize CS/DS with different properties compared to the structures found in the major sites of CS/DS deposition during zebrafish larval development.

369. Investigating molecular functions of the LINC complex in Emery-Dreifuss Muscular Dystrophy. ***Z. Li**, A. Siegel, J. Berger, P. Currie.* Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia.
Emery-Dreifuss Muscular Dystrophy (EDMD) is a degenerative disease characterized by specific muscle degeneration, joint contractures and cardiac conduction defects. Recent studies have identified the EDMD related genes encode members of LINC (LInker of Nucleoskeleton and Cytoskeleton) complex, which includes a series of nuclear envelope (NE) proteins that physically interact with each other. Besides the most overt pathological features of EDMD, such as increased muscle necrosis and connective tissue deposition, the muscle cells of EDMD patients exhibit major changes in nuclei shape and localization, chromatin organization, as well as the mislocalization of NE proteins. Consequently, two specific hypotheses have been proposed to explain how a failure of nuclear envelope function results in EDMD, though they not necessarily mutually exclusive. The “mechanical stress” hypothesis suggests that mutated LINC proteins structurally impair the integrity of the NE, causing cell death as a result of 'wear and tear' effects of the repeated force loading evident in contracting muscle cells. The second hypothesis suggests that defective nuclear envelope proteins negatively affect functions related to chromatin organization and tissue-specific transcriptional factors leading to aberrant gene regulation. So far, we have generated zebrafish models of the two most common forms of EDMD and propose to use these to determine the aberrant molecular activities in EDMD. We aim to: 1. examine in detail the pathology of the Lamin and Emerin-deficient zebrafish models we have generated; 2. compare in vivo the molecular interactions and localization of LINC components in wild type and EDMD mutant contexts using transgenic zebrafish models; and 3. investigate the differences among zebrafish models of specific human EDMD mutations.

370. Post embryotic malformation of vertebrae in zebrafish is caused by mutation of cx43. ***Akihiro Misu**, Hiroaki Yamanaka, Shigeru Kondo.* Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, Japan.
Spinal column is a central skeletal structure composed of successive vertebrae. The proportion of vertebrae is maintained and appropriately during body growth. However that mechanism has been still unknown. To elucidate it, we focused on the zebrafish mutant *stöpsel*^{dtl28d} (*stp*). *stp* is a dominant mutant. *stp* shows short vertebrae in adult fish, but *stp* embryo or larva does not show apparent phenotype. We assumed that the responsible gene of *stp* is involved in the mechanism of maintaining the proportion of vertebrae during body growth. We performed positional cloning and detect a single amino acid mutation(W78L) in cx43 in the result of positional cloning. The amino acid sequence around the W78L mutation in cx43 is highly conserved between several species. We knocked out cx43W78L allele by TALEN. This cx43W78L knockout rescued *stp* phenotype. These data indicate that post embryotic vertebrae malformation is caused by cx43W78L. Interestingly, cx43 has been already known as a responsible gene of other mutant *short fin*(*sof*). *sof* mutant has short fin ray segments but has normal vertebrae. We created *stp/sof* mutants and checked the phenotype. *stp/sof* mutant showed severly short vertebrae but normal fin ray length. It suggests that cx43W78L does not affect fin ray length in contrast with cx43^{sof}. It is very interesting that two different alleles of cx43 cause similar malformation in different bones. Cx43 has also been known as the responsible gene of human hereditary disease, ODDD (oculodentodigital dysplasia). Symptoms of ODDD are various depending on the mutated site of cx43. We expect that the mechanism of these various symptoms of ODDD would be elucidated by investigating the mechanism by which the different phenotypes of *stp* and *sof* are generated by the mutations in Cx43.

371. Pigmentation Pattern Formation in Spotted and Striped Zebrafish. ***Matilda Omoru***, *Alicia Coughlin, Jennifer Liang*. University Of Minnesotuna Duluth , duluth, MN.
In this research, we examine the development of stripes and spot pigmentation by comparing and contrasting WT fish (striped) with fish carrying the leopard mutation (spotted). We tested the hypothesis that there would be differences between the WT and leopard fish during larval and juvenile stages. This hypothesis is not supported. We found that the pigment formed complex but ordered patterns in both striped and spotted zebrafish. Further, both striped and spotted fish looked the same at 4 weeks. During seven to eight days post fertilization, both striped and spotted zebrafish had lines of dark pigment cells, melanocytes, on the dorsal, ventral and lateral sides. Some zebrafish had two lines in the lateral side while others had one. At week 7, the WT and leopard fish looked different. Both strains had two lines along the lateral sides. However, in the WT fish, the lines of melanocytes are contiguous and defined. In the mutant fish, the lines have gaps with no black pigment between cells or groups of cells. Between week 7 and adulthood, we predict that the lines in the leopard mutants will gradually disappear and will be replaced by spots.

372. Genetics and pharmacogenetics of reflexive decision-making. ***Roshan A. Jain***[1], *Marc Wolman*[2], *Kurt Marsden*[1], *Hannah Bell*[1], *Lauren Schmidt*[1], *Katherina Hayer*[1], *John Hogenesch*[1], *Michael Granato*[1]. 1) Cell & Dev Bio, University of Pennsylvania, Philadelphia, PA; 2) Dept of Zoology, U Wisconsin, Madison WI.
The nervous system constantly integrates sensory information to select appropriate, context-dependent behavioral responses. Selecting one out of several potential responses to a given situation is called decision-making. While decision-making can involve complex cognitive processing, it has become clear that even simple reflexes are dynamically biased and modulated, representing a more tractable system to study the mechanisms of decision-making. We have developed a simple reflexive decision-making paradigm in larval zebrafish using the evolutionarily conserved acoustic startle response. Larvae perform 2 kinematically and neuronally distinct forms of the startle response: a Short-Latency C-bend (SLC) initiated 4-15 ms post-stimulus, or a less vigorous Long-Latency C-bend (LLC) initiated 20-80 ms post-stimulus. Individual larvae can respond to acoustic stimuli with either behavior, yet bias their responses toward SLCs following intense (26dB) stimuli and toward LLCs following weak (13dB) stimuli. Importantly, individuals incorporate prior experience in selecting their behavioral output, shifting their response bias from SLCs to LLCs following repeated strong stimuli. Thus, the basic dynamic aspects of complex cognitive decision-making are present in the simple SLC/LLC decision-making paradigm. To identify genes and pathways critical for the development and function of startle decision circuits, we performed a small molecule screen and a forward genetic screen. The small molecule screen results demonstrate that as in more complex cognitive assays, serotonergic modulation is critical for decision-making. Through our forward genetic screen we identified 11 mutants with specific defects in SLC/LLC bias, the first vertebrate mutants specifically isolated based solely on reflexive decision-making deficits. Using whole-genome sequencing we have identified mutations in both the *calcium-sensing receptor* (*CaSR*) gene and a regulator of CaSR trafficking. We will present functional neural imaging data to elucidate how serotonergic signaling and *CaSR* gene function influence simple decision-making.

373. Employing CRISPR for Lightlight-triggered stable gene silencing and activation in zebrafish. ***K. Jiang***[1], *Y. Pi*[1], *Z. Dong*[1], *S. Qi*[2], *S. Guo*[1]. 1) Department of Bioengineering and Therapeutic Science, University of California, San Francisco, San Francisco, CA; 2) Department of Cellular and Molecular Pharmacology,University of California, San Francisco, San Francisco, CA.
Gene regulation in vivo is an essential strategy for molecular investigations of various developmental and physiological processes, which calls for stable and robust tools to silence and activate genes in a spatiotemporally controllable manner. However, such precise ways of manipulating gene activity is currently lacking in zebrafish. Light is an ideal means for precise spatiotemporal targeting. Recently, the method of CRISPR (clustered regularly interspaced palindromic repeats) has provided a convenient and efficient platform for genome editing. Here we aim to combine light regulation and CRISPR to develop precise gene silencing and activation tools. First, a CRISPR-associated catalytically inactive version of Cas9 (dCas9) and short guide (sg) RNAs targeting transcriptional repression or activation of fluorescent proteins are used. Second, light regulation of CRISPR activity is implemented. Finally, the system will be used to test the targeting of endogenous loci. This technology shall greatly enhance the utility of the zebrafish model for deciphering molecular and cellular mechanisms underlying development, physiology, and behavior.

374. Distinct genetic architecture underlies the convergence of foraging-related traits in the Mexican Cavefish. *M. Yoshizawa*[1], *B. Robinson*[1], *P. Masek*[1], *K. O'Quin*[2], *W.R. Jeffery*[3], ***A.C. Keene***[1]. 1) Dept of Biology, University of Nevada, Reno, NV; 2) Department of Biology, St. Bonaventure University, St. Bonaventure, NY 14778; 3) Department of Biology, University of Maryland, College Park, MD, 20741.
Neural regulation of sleep, appetite and energy homeostasis is critical for an animal's survival and under stringent evolutionary pressure. Across phyla, starvation results in reduced or disrupted sleep, suggesting animals suppress sleep in order to forage for food. The Mexican cavefish, Astyanax mexicanus presents a powerful system for the analysis of adaptive behavioral traits including sleep and feeding. A. mexicanus consist of eyed 'surface' populations that live in rivers and ponds throughout Mexico and 29 geographically isolated populations of cave-morphs have been identified in the Sierra Abra region of Northeast Mexico. Surface and cave forms are interfertile, representing a single species. Cave populations have adapted to survive in the dark, nutrient-poor cave environments and display a number of changes in foraging-related traits including sleep loss and vibration attraction behavior (VAB), which underlies predatory foraging; however, the genetic and evolutionary basis for these changes in foraging is unknown. We have employed behavioral and genomic approaches to investigate the genetic basis for sleep and feeding behavior. Our findings indicate that both sleep loss and VAB are present in independently derived populations of adult cavefish, providing the opportunity to examine the relationship between these foraging-related traits. Three of four independent cavefish populations showed reduced sleep suggesting the convergent evolution of this trait. Analysis of surface- cave hybrids reveals that independent genetic architecture underlies changes in sleep-wake regulation and VAB. Further, QTL analysis using restriction site associated DNA markers (Rad-Seq) in F2/F3 hybrid fish revealed distinct genomic loci underlying sleep and

VAB. Taken together, these findings demonstrate the independent convergent evolution of distinct foraging related traits and establish adult Mexican cavefish as a model for the study of evolutionarily derived sleep loss.

375. Genetic and optical approaches to understand hair-cell synapse function and development in vivo. ***Katie Kindt***[1,2], *Lavinia Sheets*[3], *Teresa Nicolson*[2]. 1) NIDCD, National Institutes of Health, Bethesda, MD; 2) Vollum Institute, Oregon Hearing Research Center, Portland, OR; 3) Mass Ear and Eye Infirmary, Boston, MA.
Hair cells, photoreceptors and bipolar cells have a specialized presynaptic density, also known as the synaptic ribbon body. The ribbon body is an electron dense structure composed primarily of a protein called Ribeye. This synaptic structure acts as a scaffold to tether vesicles adjacent to the presynaptic membrane near calcium channels ($Ca_V1.3$). At ribbon bodies, exocytosis is coupled to graded changes in membrane potential that activate synaptic calcium channels. Presynaptic ribbon bodies are able to encode the frequency, intensity and phase of stimuli. The size and shape of ribbon bodies vary depending on the requirements of a given sensory cell, but how these variations enable diverse sound encoding requirements is not clear. To study activity at ribbon synapses we have created a transgenic zebrafish that expresses a genetically-encoded calcium indicator localized exclusively to the ribbon body. This transgenic reliably measures presynaptic calcium signals. In addition we have created a transgenic that allows for measurement of vesicle release relative to presynaptic ribbon bodies. Along with these approaches to examine synapse function in vivo, we have taken advantage the zebrafish system and manipulated ribbon body genetically to understand how synapse size alters synapse function. Using this transgenic line we observe a heterogeneous range of calcium signals at individual synaptic ribbons. The calcium signals we measure are precise and local in *$ca_V1.3$* mutants we observe no calcium response at synaptic ribbons. Despite the heterogeneity in calcium signals, within a hair cell calcium signals are homogeneous. By pushing our system, either genetically or pharmacologically, we find that by increasing synapse size, we observed significant changes in the magnitude of the presynaptic calcium signal, distribution of $Ca_V1.3$, and unique changes to the properties of vesicle release. Overall this data provides new and interesting insight on the how the physical properties of synaptic ribbons can generate the diverse coding requirements in different sensory cells.

376. Functional architecture of a neural circuit underlying innate behavior in the zebrafish. ***Hernan Lopez-Schier***. Helmholtz Zentrum, Munich, Germany.
Hardwired processing of sensory input is essential for interindividual and transgenerational conservation of innate behaviors. In the zebrafish, lateral-line afferent projection neurons (LPN) that innervate sparsely distributed peripheral sensory receptors segregate into discrete populations: early- and late-born LPNs (respectively ELPN and LLPN). ELPN and LLPN present dimorphic central projections, suggesting that they have unique roles in hydromdynamic processing. However, their individual contribution to behavior has not been demonstrated. We will present evidence that reveals behaviorally-specific activities of ELPNs and LLPNs. Discretizing the LPNs ensemble may segregates synaptic inputs to the brain, allowing a single sensory modality to control distinct phases of the innate escape behavior.

377. The genetic basis of molecularly asymmetric electrical synapses in zebrafish. ***Adam Miller***, *Alex Whitebirch*, *Arish Shah*, *Cecilia Moens*. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.
Electrical synapses are specialized sites of adhesion allowing neurons to communicate directly through gap junction (GJ) channels. Both the pre- and postsynaptic neurons contribute hemichannels composed of hexamers of Connexin (Cx) protein to the GJs. Despite this apparent simplicity GJs can contain multiple different Cx proteins, which influences the functional properties of the synapse. Homotypic GJs contain identical hemichannels, while heterotypic GJs pair different Cx hemichannels on each side of the synapse. Although mammals have ~20 *cx* genes, most neuronal GJs are homotypic channels of Cx36. We found that zebrafish have four orthologous *cx36-like* genes, *cx34.1*, *cx34.7*, *cx35.1*, and *cx35.5* (gene nomenclature is under review by Zfin), but their roles in synaptogenesis are unclear. In a forward genetic screen utilizing the Mauthner (M) neuron, which forms electrical synapses on its dendrites and axon, we identified a mutant harboring two missense mutations in *cx34.1* that causes a loss of synaptic Cx36-like staining; non-complementation with a TALEN induced deletion confirmed *cx34.1's* causal role. We find that in the absence of Cx the electrical-synapse scaffolding molecule ZO-1 still localizes to sites of contact. However the mutant synapses are functionally defective. To examine Cx34.1's contribution to synapses we generated chimeric fish in which postsynaptic neurons in the M escape circuit were *cx34.1* mutant while presynaptic neurons were wildtype (wt) and found a loss of synaptic Cx36-like staining. Conversely, staining is retained when the postsynaptic neuron is wt and the presynaptic neuron is mutant. We conclude that these neuronal GJs are heterotypic and only the postsynaptic hemichannels contain Cx34.1 where it is required to stabilize the GJ. Furthermore another Cx36-like protein is present presynaptically. Why would a neural circuit use asymmetric GJs? Heterotypic electrical synapses have been shown in invertebrates and in goldfish M synapses to mediate rectified, i.e. unidirectional, signaling through the GJ - in the case of goldfish M, the rectification allows for the potentiation of signaling ensuring a robust escape response.

378. Modulation of Larval and Adult Zebrafish Behavior by Maternal Rest. ***Cara Moravec***[1,2], *Hans Maaswinkel*[3], *Wei Weng*[3], *Howard Sirotkin*[1,2]. 1) Neurobiology and Behavior, Stony Brook University, Stony Brook, NY 11794; 2) Genetics Graduate Program, Stony Brook University; 3) xyZfish, 2200 Smithtown Ave, Ronkonkoma, NY 11779, USA.
Changes to the chromatin landscape early in development can have long-term impacts on behavior. The RE1-silencing transcription factor (Rest) transcriptional repressor interacts with chromatin-modifying complexes to repress transcription of neural specific genes. In zebrafish, rest is maternally expressed, and the chromatin modifications mediated by Rest have the potential to exert long-term effects on gene regulation. We have previously shown that maternal rest is required for early repression of target genes. We have now discovered that the elimination of maternal rest has behavioral effects in both larval and adult fish. To eliminate maternal rest, we crossed rest mutant females to wild-type males, creating a line of fish that lack maternal rest but have zygotic rest (Mrest). At 6dpf, Mrest larvae show an increase in both spontaneous movements and thigmotaxis when compared to controls. No differences in evoked movement in response to a

light change were observed between the Mrest larvae and controls, indicating that the Mrest larvae have similar swimming kinetics as controls. Therefore, we conclude that the hyper-locomotion phenotype is not due to a musculoskeletal abnormality. Rest mutants that have maternal rest but lack zygotic rest do not show hyper-locomotion or increased thigmotaxis when compared to the sibling controls. This suggests that the maternal and zygotic roles for Rest differ, and the change in locomotive behavior stems from the elimination of maternal rest. To examine the long-term requirement for maternal rest, we investigated behavior at 6 months with novel environment and mirror-induced aggression test. In the novel environment assay, Mrest males but not females show increased thigmotaxis when compared to the controls. In the mirror induced aggression test, Mrest males displayed more aggression towards the mirror than the controls. Based on these observations, we conclude that maternal rest not only plays a role in repressing expression of target genes at early stages, but also has an essential function in modulation of both larval and adult behaviors.

379. An endoplasmic reticulum-resident ubiquitin ligase regulates the transport of voltage-gated sodium channels in zebrafish sensory neurons. ***K. Ogino***[1], *S. Low*[2,3,4], *K. Yamada*[1], *L. Saint-Amant*[4], *W. Zhou*[4,5], *W. Cui*[4,6], *A. Muto*[7,8], *K. Asakawa*[7,8], *J. Nakai*[9], *K. Kawakami*[7,8], *J. Kuwada*[4], *H. Hirata*[1,8,10]. 1) Center for Frontier Research, National Institute of Genetics; 2) Howard Hughes Medical Institute; 3) The Rockefeller University; 4) Department of Molecular, Cellular and Developmental Biology, University of Michigan; 5) Department of Pediatrics, University of Michigan; 6) Department of Anesthesia and Perioperative Care, University of California, San Francisco; 7) Division of Molecular and Developmental Biology, National Institute of Genetics; 8) Department of Genetics, Graduate University for Advanced Studies; 9) Brain Science Institute, Saitama University; 10) PRESTO, JST.
Following their biosynthesis in the endoplasmic reticulum, voltage-gated sodium channels (Nav) are transported to the membranes of neurons where they are clustered at the axon initial segment and nodes of Ranvier. Although the mechanism by which Nav channels are clustered has been extensively studied, the process that governs their transport to the membrane remains largely unknown. To gain insight into this process we screened for touch-unresponsive mutants, since a loss of Nav channel activity often results in unresponsive larvae. One such mutant, rnf121 have a defect in the proper transport of Nav channels to the axon initial segment of sensory neurons. The rnf121 gene encodes an endoplasmic reticulum-resident ubiquitin ligase, which mediates ubiquitination of Nav1.6 for proteasome-dependent degradation. Our findings suggest that RNF121-dependent quality control of Nav1.6 is a necessary process for zebrafish's responsiveness to sensory stimulation.

380. Modeling human developmental disorders in zebrafish. ***Sebastian Gerety****, Eve Coomber, Margriet van Kogelenberg, Matthew Hurles, on behalf of the DDD project.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.
Although individually rare, developmental disorders taken together affect approximately two percent of live births, and are a major cause of infant mortality and morbidity. Deciphering Developmental Disorders is a collaborative study aimed at identifying the underlying genomic changes in a large cohort consisting of 10,000 children in the U.K. with undiagnosed severe developmental malformations. The project uses Copy Number Variation assessment and whole exome sequencing of patient-parent trios to identify inherited or de novo genetic changes. 1133 patient/parent trios have been sequenced to date. Known disease causing mutations are reported back to referring clinicians, for verification and communication to the affected patients. To explore the consequences of novel genetic changes during development, the remaining candidate mutations are subjected to a number of analyses to identify likely causal changes based on mutation consequence, recurrence in our cohort, protein interaction networks, and published animal models. From these, we selected a set of 35 genes with no existing animal models in which we found loss of function mutations in our 1133 trio cohort. We performed a morpholino antisense-based loss of function, and compared larval zebrafish phenotypes to the clinical phenotypes in patients. The results of this screen will inform further work in zebrafish, mouse, and cellular systems to elucidate the biological mechanisms underlying these disorders. We will present our methodology, progress to date, and discuss the application of zebrafish as a model organism in validating human genetics findings.

381. ECM Modifications Guide Pathway Selection in Peripheral Nerve Regeneration. ***Jesse Isaacman-Beck****, Michael Granato.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
Following injury, peripheral nerves maintain the remarkable ability to reestablish functional connections. Trophic factors such as BDNF and NGF provide critical and well-defined signals to spur regrowth, but the molecular cues that return these nerves to the correct pathway at choice points remain largely unknown. We use the larval zebrafish peripheral motor nervous system to determine these regenerative guidance signals. In these larvae, all motor nerves exit the spinal cord and initially fasciculate along a common ventral path. Just prior to the horizontal myoseptum, a subpopulation of axons turns acutely from this shared corridor and extends to innervate the dorsal myotome. After we transect both dorsally and ventrally projecting nerves, regenerating axons from both nerves navigate to their original synaptic targets, suggesting that cues exist to guide this binary pathway selection. Here, we provide real-time *in vivo* evidence that constituents of the extracellular matrix (ECM) govern pathway selection for peripheral motor nerve regrowth. In mutants lacking the ECM glycosyltransferase *lysyl hydroxylase 3 (lh3)*, ventral motor nerves regrow robustly to the ventral myotome, but dorsal nerves fail to stabilize growth to dorsal targets. When we analyzed dorsal nerve regrowth in null mutants for three *lh3* substrates: *collagen4a5 (col4a5)*, *collagen18a1 (col18a1)*, and *collagen19a1 (col19a1)*, we found that while dorsal nerve regrowth is similar to wild type in *col18a1* and *col19a1* mutants, regrowing dorsal nerve axons in *col4a5* mutants frequently extend along ectopic trajectories, invading lateral and ventral territories. Moreover, larvae deficient in the canonical axon guidance receptor *robo2* show similar pathway selection defects in nerve regrowth. Interestingly, Col4a5 is known to bind Slit with high affinity to facilitate *slit-robo2* guidance of axons. We will link these findings with data that suggest that after injury, *lh3* acts in nerve associated glia, modifying the ECM to provide *robo2*-mediated guidance for dorsal pathway selection. To our knowledge, this is the first direct evidence defining a functional mechanism for ECM cues in establishing pathway selection in peripheral nerve regrowth *in vivo*.

382. Souffle/Spastizin Regulates Dense Core Vesicle Maturation:A Novel Mechanism for Hereditary Spastic Paraplegia (HSP). ***Palsamy Kanagaraj***[1], *Amandine Gautier-Stein*[2], *Dietmar Riedel*[3], *Joan Cerda*[4], *Roland Dosch*[1]. 1) Dept. of Developmental Biochemistry, Georg-August-Univ Goettingen, Goettingen, Germany; 2) Department de Zoologie et Biologie Animale, Universite de Geneve, Switzerland; 3) Max-Planck Institut fuer Biophysikalische Chemie, Goettingen, Germany; 4) IRTA - Institute of Marine Sciences, (CSIC), Barcelona, Spain.
Vesicle transport is very active during oogenesis of zebrafish. Hence, oocytes are an excellent system to study the regulators of vesicle trafficking. Here, we combine zebrafish genetics and cell biology in the oocyte model to study the role of souffle (suf) mutation. We show that suf encodes a homolog of the gene SPASTIZIN (SPG15), which causes the neurodegenerative disease Hereditary Spastic Paraplegia (HSP) in humans. We find that suf mutants accumulate immature secretory granules and fail to form mature dense core vesicles (DCV) describing a novel role for Suf. Electron microscopy and immunostaining revealed that Suf is necessary for secretory granule maturation into secretion-competent DCV. Further analysis showed that suf mutant oocytes have a defect in sorting and fission from ISG. Interestingly, suf mutants also accumulate non-functional fragmented lysosomes, which explain the observed opaque egg phenotype. The analysis of lysosomal cargo suggests that Suf is necessary for sorting from ISG into lysosomes, since suf mutants accumulate their cargo in ISG. This result connects the defect in DCV formation and lysosomes. Interestingly, sorting by Suf seems to be required upstream of Dynamin, which regulates vesicle fission. Inhibition of dynamin mimics the suf phenotype at the cellular and morphological level, supporting its role in sorting lysosomal cargo from ISG, which leads to DCV formation. Consistent with these data our bioinformatic analysis suggests that Suf might function as a coat protein. Taken together, our results report an uncharacterized cellular mechanism for Suf in sorting lysosomal cargos from ISG and maturation of secretion competent DCV. Moreover, these data connect of the first time the secretory pathway to lysosomal disorders and hence, open a novel research direction for neurodegenerative diseases such as HSP.

383. Functional Roles of A Telomeric Factor TRF2 in Early Vertebrate Development. *Shanshan Lian*[1], *Tomoyuki Sasaki*[1], *Jie Qi*[1], *Michael Rebagliati*[2], *Laure Bally-Cuif*[3], *Howard Sirotkin*[4], ***Shuji Kishi***[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Institute of Genetics and Molecular and Cellular Biology, France; 3) Institute of Neurobiology Alfred Fessard, France; 4) Stony Brook University, Stony Brook, NY.
Telomere repeat-binding factor 2 (TRF2) is critical for telomere integrity in dividing stem and somatic cells, and its role is essential for vertebrate early development because animals carrying a null *terf2* mutation have early embryonic lethality due to an unknown molecular mechanism. Besides telomeric homeostasis by protecting telomere ends, TRF2 can interact with the neuronal gene-silencer repressor element 1-silencing transcription factor (REST), whose instability via TRF2 affects neuronal cell differentiation in vitro. However, the mechanism that couples TRF2-dependent features to cellular differentiation programs has yet to be elucidated *in vivo*. Loss of TRF2 in zebrafish embryos recapitulates key aspects of telomere attrition, including the DNA-damage response and cell-cycle arrest, as well as neurodegeneration. Spinal muscular atrophy (SMA) is a genetic disorder characterized by a loss of alpha motoneurons in the spinal cord. Intriguingly, TRF2-deficient animals develop similar but more severe neuromuscular defects. Using *terf2* and *rest* mutant zebrafish embryos expressing *islet-1:GFP*, *gata2:GFP*, or *her5:GFP* transgenes, we found motor axon-specific pathfinding/guidance abnormalities. Our results show for the first time, in vivo, that TRF2 functions in motor axon development and suggest that *terf2* mutation leads to early developmental motoneuron defects.

384. Mechanisms of Purkinje Cell Degeneration in Menkes Disease. ***Andrew Latimer***[1], *Victoria Hodgkinson*[2], *Michael Petris*[2], *Jonathan Gitlin*[1]. 1) Marine Biological Laboratory, Woods Hole, MA; 2) Department of Biochemistry, University of Missouri, Columbia, MO.
Copper is an essential nutrient that plays a critical role in neuronal development, as evidenced by Menkes disease, a neurodegenerative disorder resulting from inherited loss-of-function mutations in the gene encoding the copper-transporter ATP7A. *calamity*, a mutant defective in zebrafish *atp7a*, demonstrates neurodegeneration of the midbrain-hindbrain region, analogous to the gray matter degeneration and neuronal loss in the cerebellum of patients. High resolution imaging reveals normal midbrain-hindbrain development in *calamity* embryos up to 36hpf, after which the cerebellar primordium becomes progressively smaller compared to wild-type siblings. *In situ* hybridization with molecular markers for cerebellar neuronal progenitors reveals no differences in cell number or patterning, suggesting that copper deficiency directly affects the survival of differentiated neurons. Consistent with this idea, live cell confocal imaging utilizing an *aldoca:mGFP* transgene specific for Purkinje cells reveals decreased numbers of Purkinje cells within the cerebellum of *calamity* embryos. Identical results are observed with copper chelation, highlighting the unique sensitivity of Purkinje cell survival to copper limitation. To determine the mechanisms of Purkinje cell neurodegeneration, transplantation experiments were performed utilizing wild type and *calamity aldoca:mGFP* transgenic fish as donors. Wild type Purkinje cells transplanted into calamity hosts demonstrate progressive degeneration over the first 7 days. In contrast, *calamity* Purkinje cells transplanted into wild type hosts survive and develop normally, demonstrating robust dendritic arborization at 7dpf. Consistent with these results, mice with a Purkinje-cell specific deletion of *Atp7a* reveal normal cerebellar development with no apparent Purkinje cell loss or abnormalities. These studies demonstrate a non-cell autonomous mechanism of Purkinje cell degeneration in Menkes disease and reveal a unique mechanism for copper in neuronal development, suggesting a novel approach to prevent cerebellar neurodegeneration in affected patients.

385. A screen for factors that modulate spinal cord regeneration in zebrafish. ***Mayssa H. Mokalled****, Kenneth D. Poss.* Cell Biology, Duke University Medical Center, Durham, NC.
Developing means to treat and reverse spinal cord (SC) injury remains a pressing need in regenerative medicine. To date, advancing efficient cures for SC injury has been hindered by the limited ability of mammals to regenerate neurons and establish functional connections following damage. Unlike mammals, zebrafish are capable of efficient, spontaneous recovery after SC injury. As a result, adult zebrafish provide a unique platform to dissect the molecular mechanisms of axon regeneration and stem cell-based neurogenesis. To elucidate these mechanisms, we used transcriptome profiling and in situ hybridization screening to identify candidate genes that could

promote SC regeneration. Our screen identified unique spatial and temporal expression patterns for multiple extracellular, signal-transducing growth factor molecules. Several candidates showed specific expression within subdomains of ependymal cells, suggesting that ependymal cells perform specialized functions during SC regeneration. To examine the roles of candidate factors during SC regeneration, we are treating injured fish with human recombinant proteins and assessing their morphological and functional recovery. Additionally, we are developing transgenic and loss-of-function tools to dissect the functions of our screen hits in glial bridging, neural stem cell activity, and axon regeneration.

386. Molecular Mechanism that Converts Vesicular Fusion Defects into Apoptosis in Photoreceptors. ***Y. Nishiwaki****, S. Nakamura, E. Oguri, M. Araragi, Y. Kojima, I. Masai.* Okinawa Institution of Science and Technology, Onna-son, Kunigami-gun, Okinawa, Japan.
Defects in intracellular protein transport induce photoreceptor degeneration in human. Intracellular protein transport is mediated by vesicular transport system. However, it is unclarified how vesicular transport defects are linked to photoreceptor degeneration. We reported that mutations of a vesicular fusion regulator, b-SNAP, cause photoreceptor degeneration, which depends on BH3-only SNARE, BNip1. BNip1 is a SNARE component of syntaxin18 complex, located on ER membrane, and mediates retrograde transport from Golgi to ER. BNip1 interacts with Bcl2 by its BH3 domain and induces Bax-dependent apoptosis by releasing Bax from Bcl2-mediated suppression. In our model, b-SNAP mutation inhibits disassembly of syntaxin18 cis-SNARE complex, which is generated after vesicular fusion. Accumulation of syntaxin18 cis-SNARE complex subsequently activates BNip1 BH3-domain to induce Bax-dependent apoptosis. However, it remains to be elucidated how activated BNip1 on the ER membrane induces Bax-dependent apoptosis. Bax activates apoptosis by increasing mitochondrial outer membrane permeabilization. Bcl2 is located in both mitochondria and ER membranes. To elucidate whether BNip1 interacts with Bcl2 on the ER, we designed ER membrane-targeted Bcl2 (ER-Bcl2) and drove its expression in zebrafish b-SNAP mutant. We found that ER-Bcl2 significantly inhibited photoreceptor apoptosis in b-SNAP mutant, suggesting that the interaction of BNip1 with Bcl2 on the ER is the first step of photoreceptor apoptosis in response to vesicular fusion defects. Taken together, these data suggest that formation of BNip1-Bcl2 complex, which follows the accumulation of syntaxin18 cis-SNARE complex, is a central component of molecular mechanism that converts vesicular fusion defects into apoptosis.

387. The chemotherapeutic agent paclitaxel induces keratinocyte damage and sensory axon dysfunction prior to axon degeneration. ***Sandra Rieger****, Emily Spaulding, Erin Carter.* Regenerative Biology and Medicine, MDIBL, Salisbury Cove, ME, USA.
Chemotherapy is a common cancer treatment and although chemotherapeutic agents arrest tumor growth, they also attack healthy tissues due to undefined, non-specific effects. The chemotherapeutic agent paclitaxel (PTX) is used to treat the most prevalent cancers, including breast, ovarian and lung cancer. It is estimated that up to 94 % of PTX-treated patients develop mild to severe PTX-induced peripheral neuropathy (PIPN), the degeneration of peripheral axons, which is dependent on the rate and dose of administered PTX. Although many patients recover from PIPN, chemotherapy must be aborted when severe symptoms develop, thus representing a life-threatening condition. PTX is a microtubule-stabilizing agent and in cancer cells prevents cell cycle transition from G2 to M-phase. Its side effects in neurons are less well understood. PIPN initiates in the epidermal layers of the palms and soles, which undergo increased mechanical stress. Thus the skin may play a critical role in PIPN. To test this, we developed a model for observing PTX-induced cellular changes with *in vivo* imaging in larval zebrafish. We found that PTX selectively induced PIPN in the larval tail fin. To verify this result in adult animals, we injected PTX 4x daily into the peritoneal cavity. Similar to the larval phenotype, immunohistochemistry revealed a selective loss of sensory fibers in the adult tail fin. To further analyze PTX effects in keratinocytes, we stressed Tg(*NF-kB-GFP*) larvae with gentle pipetting. While a stress response was mildly activated in DMSO controls, PTX-treated larvae significantly increased NF-kB activity and displayed lacerations to the skin. To further characterize PTX effects in sensory axons we assessed their ability to regenerate upon tissue injury, which we previously showed is hydrogen peroxide (H_2O_2)-dependent. Interestingly, brief PTX treatment prevented H_2O_2 production and also inhibited injury-dependent sensory axon regeneration. To further unravel the molecular mechanisms underlying PIPN and identify novel compounds with therapeutic potential for PIPN treatment, we will implement a pharmacological screening assay. In summary, our data suggest that keratinocytes play an important role in PIPN development.

388. Small molecule modulators of Erv1 impair neuronal branching and mitochondrial movement in primary motor neurons during development. ***C. Koehler****, M. Johnson.* Dept Chem/Biochem, Young Hall, Univ California, Los Angeles, Los Angeles, CA.
The mitochondrial disulfide relay system, consisting of Mia40 and Erv1, mediates the import of small Tim proteins and other cysteine-rich proteins within the intermembrane space (IMS). Erv1 and Mia40 mediate the insertion of disulfide bonds into substrates. Mutations in Erv1 lead to neuropathy in adults, suggesting Erv1 plays an important role in the neural system. We therefore used a small molecule modulator (MitoBloCK-6/MB-6) that inhibited Erv1 function to determine the role of the redox-regulated import pathway in development of primary motor neurons. MB-6 treatment resulted in a defect in neuronal growth and branching. Moreover, mitochondrial movement in the neurons was markedly inhibited. Because dysfunctional Erv1 can generate ROS, we considered this as a potential mechanism that caused the defect in neuronal development. Indeed, treatment with the ROS scavenger N-acetyl cysteine reversed the developmental defects caused by MB-6 treatment in motor neurons. Therefore, this study supports an important role for redox-regulated protein import in the development of the neuronal system.

389. Calsyntenin-1 affects microtubule dynamics during axon development in vivo. ***Tristan J. Lee****, Olga Y. Ponomareva, Mary C. Halloran.* Neuroscience Training Program, University of Wisconsin-Madison, Madison, WI.
Axon formation, branching, and guidance are crucial to the formation of functional neural circuits. Precise regulation of microtubule (MT) dynamics is essential for these processes, yet the mechanisms that regulate MT dynamics during axonal development are not well understood, especially in vivo. We are investigating MT dynamics during sensory neuron axon development using live imaging with an EB3-GFP fusion protein in vivo. Sensory neurons extend separate peripheral and central axons with distinct trajectories and branching

behaviors. We found that MTs polymerize faster in actively growing and branching peripheral axons compared to growing central axons, suggesting differences in MT dynamics may underlie differential axon behavior. Moreover, we find that MTs invade only limited regions of peripheral growth cones and these invasions predict subsequent branching patterns and growth direction. Interestingly, this behavior differs from reported behaviors in cultured neurons, in which MTs invade broad regions of growth cones. This difference suggests growth cones in vivo are actively responding to graded guidance information and that selective MT invasion is an early response to cues. We also investigated the role of Calsyntenin-1 (Clstn1) in regulating MT dynamics. Clstn1 is a kinesin adaptor that we found is required specifically for peripheral axon formation and branching. In Clstn1 knockdown embryos, MT polymerization rate is markedly reduced in peripheral axons, suggesting that Clstn1 affects axon initiation and branching by regulating MT dynamics.

390. Timing and range of temperature effects on neural tube development of oepm134 mutants. ***Phyo M Ma***, *Morgan Swartz, Lexy Kindt, Jennifer O Liang.* Biology, University of Minnesota Duluth, Duluth, MN.
Neurulation is the process of neural tube closure. A Nodal signal, Squint (Sqt/Ndr1), and a protein in the Nodal receptor complex, One eye-pinhead (Oep), are required for proper neural tube closure in zebrafish embryos. Interestingly, the open neural tube phenotype in both sqt and oep mutants is incompletely penetrant, only a subset of homozygous mutants have open neural tubes. Research on sqt mutants has shown that temperature impacts the penetrance of the mutant phenotype. Normal temperature conditions for zebrafish embryos are around 28.5 ºC. Pei and colleagues (2007) found that subjecting sqt mutants to a 34 ºC heat shock overnight resulted in a marked increase in the cyclopic eye phenotype. Since Oep is part of the same pathway as Sqt, we hypothesized that temperature also affects oep mutants. To test this, we raised half of each clutch of oep mutants at 28.5 °C and 34 °C and then assayed them for an open anterior neural tube. The mutant oepm134/ oepm134 embryos raised at 34 °C expressed neural tube defect phenotypes at statistically higher frequencies than embryos raised at 28.5 °C. Pei and his colleagues also found that the timing of exposure to the increased temperature was important. Embryos moved to higher temperatures before or during blastula stages had increased cyclopia and increased lethality. Similarly, we found that embryos transferred to 34°C between 0-4 hpf (one cell stage to sphere stage) were more affected by the higher temperature than embryos transferred at later stages. As in the sqt studies, we also found an effect of genetic background. In most cases, the penetrance of the open neural tube phenotype was more similar between oep embryos from the same clutch than between embryos from different clutches. The penetrance and severity of many phenotypes are affected by multiple factors. sqt and oep mutants may serve as a valuable model for studying the interactions between genetic environment and external environment during embryogenesis.

391. Ties between development and function in migrating branchiomotor neurons. ***Kimberly L. McArthur***, *Joseph R. Fetcho.* Cornell University, Ithaca, NY.
A neuron's place of birth plays an important role in establishing early synaptic connections, and predicts later key functional attributes. However, many neurons migrate after their birth, sometimes across long distances. Understanding how neural circuits form in the context of migration - and how the brain compensates when migration fails to occur normally - will shed light on fundamental principles guiding early organization of the nervous system. In wild type zebrafish embryos, facial branchiomotor neurons (FBMNs) in the hindbrain undergo a dramatic caudal migration from their birthplace in rhombomere 4 to rhombomeres 6 and 7. These neurons innervate muscles involved in feeding and respiration, making them and their associated circuitry critical for survival. We have studied the relationship between developmental patterns and functional properties of post-migratory FBMNs in wild type zebrafish. Even after caudal migration, FBMNs adopt a topographic arrangement by age along the dorsoventral axis, similar to that previously described for hindbrain interneurons and spinal motor neurons. Further, mapping the positions of discrete motor pools within the facial nuclei provides evidence that each motor pool contains both old and young neurons. Finally, FBMNs exhibit two broad patterns of activity, apparent in both calcium imaging and whole cell recordings. At 5 days post-fertilization, all FBMNs burst during swimming bouts, and a subset of these also burst spontaneously at approximately 1Hz - likely driving spontaneous movements of the operculum. Our work shows that developmental order is maintained by migrating neurons once they reach their destination, and provides baseline data for future studies of how this order and neuronal functional properties are maintained or disrupted in mutants with migration defects.

392. Interaction among 16p11.2 genes reveals key candidates affecting brain development, neuron patterning, and the enteric nervous system. ***Jasmine M. McCammon***[1], *Alicia Blaker-Lee*[1], *Chelsea Chen*[1,2], *Hazel Sive*[1,2]. 1) Whitehead Institute for Biomedical Research, Cambridge, MA; 2) Massachusetts Institute of Technology, Cambridge, MA.
Our goal is to identify pivotal genes in the 16p11.2 copy number variant region (CNV), a ~600 kb region of the genome where duplication or deletion is associated with autism spectrum disorders. A range of co-morbid pathologies is also associated with this CNV, including intellectual disability, developmental delay, language disorders, hypotonia, seizures, macrocephaly, obesity, gastrointestinal problems, and brain birth defects. The association of these issues with variable 16p11.2 copy number suggests that changes in gene dosage and gene expression within the region is integral to this connection. Because no variants of single genes from 16p11.2 have emerged, we hypothesize that two or more genes from this interval interact to confer copy number sensitivity. The multitude of genetic interactions that can arise from the 25 core genes in 16p11.2 is most efficiently studied in the zebrafish. Our focus is to associate molecular and cellular phenotypes with changes in gene copy number, a strategy that will be crucial in patients for unbiased diagnosis of each disorder and of co-morbidities. We have analyzed 162 pair-wise interactions of 16p11.2 homologs to determine whether they impact brain development in the zebrafish, and 15 pairs have emerged as being dosage-sensitive in a synergistic manner. All interactions discovered are novel, and they could not have been predicted based on known gene function. Genes that interact to alter brain development include: *asphd1*, *fam57b*, *hirip3*, *kctd13*, and *kif22*. A subset of these interactions also affect *islet1:GFP* and *NeuroD:GFP* expression in transgenic lines, as well as HuC expression in the enteric nervous system. Each gene pair displays a unique phenotypic “signature” indicating that the various phenotypes are under complex genetic control that can be defined in the zebrafish. The study thus uses accessible and powerful zebrafish tools to address mechanisms underlying a major set of neurodevelopmental disorders.

393. Mauthner-homolgous reticulospinal neurons are the initially appearing glycine-immunoreactive neurons in the embryonic zebrafish brain. ***Pricila K Moly***[1,2], *Takanori Ikenaga*[1,3], *AFM T Islam*[1,2], *Chihiro Kamihagi*[1], *Kohei Hatta*[1]. 1) Graduate School of Life Science, University of Hyogo 3-2-1 Kouto, Kamigori, Ako-gun, Hyogo 678-1297. Japan; 2) Cellular and Molecular Medicine, University of Arizona, 1656 E. Mabel, AZ 85724, USA; 3) Department of Chemistry and Bioscience, Graduate School of Science and Engineering, Kagoshima University, 1-21-35 Korimoto, Kagoshima-shi, Kagoshima 890-0065, Japan.
Glycine is a one of the major inhibitory neurotransmitter in the central nervous system of vertebrates. In this study, we investigated the initial development of glycine-immunoreactive (Gly-ir) neurons and fibers in zebrafish. By immunostaining it was found that the earliest Gly-ir cells were observed in the hindbrain and rostral spinal cord by 20 hours post-fertilization (hpf). Fluorescence in situ hybridization showed that Gly-ir cells in rhombomeres 5 and 6 in the hindbrain also expressed glycine transporter 2 (glyt2) mRNA, suggesting that they are glycinergic. These neurons were highly stereotyped and they were bilaterally located and their axons ran across the midline and gradually turned caudally, joining the medial longitudinal fascicles in the spinal cord by 24 hpf. Gly-ir neurons in rhombomere 5 were uniquely identified, since there was one per hemisegment, whereas the number of those in rhombomere 6 were variable from one to three per hemisegment. Labeling of these neurons by single-cell electroporation and tracing them until the larval stage revealed that they became MiD2cm and MiD3cm respectively. The retrograde labeling of reticulo-spinal neurons in Tg(glyt2:gfp) larva, which express GFP in Gly-ir cells, and a genetic mosaic analysis with glyt2:gfp DNA construct also supported this notion. Gly-ir cells were also distributed widely in the anterior brain by 27 hpf, whereas glyt2 was hardly expressed. These results provide evidence of identifiable glycinergic (Gly-ir/glyt2-positive) neurons in vertebrate embryos, and they can be used in further studies on the development and function of the glycinergic neurons at the single-cell level.

394. The adhesion-GPCR Gpr56 is required for oligodendrocyte development. *Sarah DeGenova, **Kelly R. Monk**.* Department of Developmental Biology, Washington University School of Medicine, Saint Louis, MO.
Myelin is a multilamellar membrane that insulates axons in the vertebrate nervous system to facilitate rapid action potential propagation. In the central nervous system, oligodendrocytes form myelin by extending and iteratively wrapping their plasma membranes around axons, and oligodendrocyte cytoplasm is extruded to ultimately form the compact myelin sheath. Impaired myelination is the cause of many severe neurological disorders such as multiple sclerosis, and myelin disruption can lead to neuronal loss and eventual paralysis. Despite the importance of myelin in human health and disease, the molecular mechanisms that regulate oligodendrocyte development and myelination are incompletely understood, although several studies have implicated extracellular matrix proteins and their receptors as important regulators of these processes. The family of adhesion G protein-coupled receptors (aGCPRs) is a major class of proteins that mediates cell-matrix interactions, and thus could play important roles in oligodendrocytes. Here, we use zebrafish to define the function of Gpr56, an aGPCR, in oligodendrocyte development and myelination. We show that *gpr56* is expressed in early stages of oligodendrocyte development, but not later stages. We utilized TALENs to generate targeted mutations of *gpr56* in zebrafish; thus far, we have recovered two mutant alleles. Using marker analysis, electron microscopy, and time-lapse imaging, we show that *gpr56* mutants have multiple defects in oligodendrocyte development and myelination. We observe a significant reduction of mature oligodendrocyte number and of myelination in *gpr56* mutants, but other CNS cell populations are unaffected. Our analysis indicates that this reduction is due to decreased proliferation, but not a result of increased cell death or altered neural precursor differentiation potential. Finally, we provide evidence that these functions of Gpr56 are mediated via interactions with $G\alpha_{12/13}$ proteins and Rho activation. Together, our studies define the aGPCR Gpr56 as a new regulator of oligodendrocyte development and myelination.

395. Morphological changes in intraspinal serotonergic neurons during larval zebrafish development. ***Jacob E. Montgomery***[1], *Timothy D. Wiggin*[1], *Brittany Corwin*[1], *Christina Lillesaar*[2], *Laure Bally-Cuif*[2], *Mark A. Masino*[1]. 1) Department of Neuroscience, University of Minnesota, Minneapolis, MN; 2) Zebrafish Neurogenetics, N&D CNRS, Gif-sur-Yvette, France.
In vertebrates, much of the neural circuitry that is required for coordinated locomotion is contained within the spinal cord. The output of this circuitry is modulated by serotonin, which is derived from two distinct sources in vertebrates; 1) descending projections originating in the raphe and 2) a population of intraspinal neurons. The number and distribution of the intraspinal serotonergic neurons (ISNs) vary between species, as mammals possess a relatively small number of ISNs when compared to fish and amphibians. Although the functional properties of the ISNs have not been established in zebrafish, morphological evidence suggests that they may innervate motor neurons, thus examination of ISN development will likely provide insight into the role of 5-HT in establishing mature locomotor behavior.
A 3.2kb fragment of the zebrafish *pet1* promoter was used to drive expression of fluorescent reporters in the ISNs of developing zebrafish larvae. Confocal imaging of EGFP expression *in vivo* was used to quantify ISN cell number and location along the rostro-caudal and dorso-ventral axes of the spinal cord. We selectively photoconverted single Kaede-expressing ISNs with a UV laser to visually isolate and characterize individual neurons. Projection distance, neurite length, and arborization were measured in photoconverted ISNs and tracked from 3-10 days post fertilization (dpf). ISN cell number and morphological characteristics exhibited the greatest changes between 3 and 4 dpf. The timing of these morphological changes corresponds with the switch in zebrafish swimming behavior from long, infrequent swimming episodes at 3 dpf to shorter, more frequent swimming episodes at 4 dpf. Future work will test the functional maturation of the ISNs as well as their involvement in modulating locomotor activity.

396. Characterization of the highly conserved m2de4 non-coding element associated with the *Meis2* gene. ***Tucker M. Munday***[1], *Kyle Nelson*[2], *Ted Zerucha*[1]. 1) Biology, Appalachian State University, Boone, NC; 2) Wake Forest University Graduate School, Molecular and Cellular Biosciences, Winston-Salem, NC.
Highly conserved non coding elements (CNEs) are thought to play important roles in gene regulation. Some CNEs have been shown to function as enhancers, directing spatial and temporal gene expression during embryonic development. The Meis genes play important roles during development as their protein products act as transcription factors and as cofactors, for example with Hox proteins. The Zerucha lab

has identified four putative CNEs associated with the Meis2 gene in vertebrates that we have named m2de1-4 (for Meis2 downstream element). While all four CNEs have been identified in tetrapods, to date teleosts appear to only have one. The purpose of this study is to characterize the regulatory role of one of the tetrapod CNEs, m2de4 using zebrafish as a model system. Making use of the Tol2 system, preliminary evidence indicates that the m2de4 element is able to direct reporter gene expression to various neurons located primarily in the developing midbrain and hindbrain. This expression pattern is consistent with the reported expression pattern of Meis2 in developing mouse embryos.

397. Using Larval Zebrafish Behavioral Assessment to Screen for Mammalian Developmentally Neurotoxic Chemicals. ***S. Padilla***[1], *M. Culbreth*[1,2], *R. C. MacPhail*[3], *D. L. Hunter*[1], *K. Jarema*[1], *K. Jensen*[1], *J. Olin*[1], *A. Tennant*[1]. 1) NHEERL, ORD, U.S. EPA, RTP, NC, USA; 2) Albert Einstein College of Medicine, Bronx, NY, USA; 3) VA Tech, Blacksburg, VA, USA.
The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals for developmental toxicity. As such, we are exploring a behavioral testing paradigm, which can assess the effects of sublethal and subteratogenic concentrations of developmental neurotoxicants on 6 day larval zebrafish (*Danio rerio*). This assay simultaneously tests individual zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and intensity of light, we are able to detect changes in locomotion during light-dark transitions, and adaptation to both light and dark during the approximate 1.5 hour testing period. Multiple chemicals at several concentrations (£ 120 mM nominal concentration) can be tested in large numbers of larvae using this method. We have evaluated a training set of chemicals (n=21) that are generally considered positive (n=15) or negative (n=6) controls for developmental neurotoxicity in mammals. Many of the developmentally neurotoxic compounds perturbed behavior at subteratogenic doses (e.g, lead, heptachlor, chlorpyrifos, chlorpyrifos oxon), while many non-neurotoxic compounds did not (e.g., acetaminophen, saccharin, glyphosate). Exposure to developmental neurotoxicants altered the overall activity level in light and dark conditions, and/or the activity pattern. The zebrafish neurodevelopmental assay using this training set of chemicals had a sensitivity of 0.93 and a specificity of 0.86. The training set results, therefore, indicate that careful evaluation of zebrafish larvae behavior is capable of identifying mammalian developmental neurotoxicants. *This abstract may not necessarily reflect official Agency policy.*.

398. Islr2 interacts with Vasorin and regulates retinotectal axon pathfinding. ***Paolo Panza***[1], *Hans-Martin Maischein*[1], *Gavin J. Wright*[2], *Christian Söllner*[1]. 1) Department 3/Genetics, MPI for Developmental Biology, Tübingen, Germany; 2) Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.
Because pathways for axon guidance are sensed by growth cones through transmembrane proteins, we took an unbiased approach to reliably detect highly transient binding between cell-surface molecules. By taking advantage of the AVEXIS assay, we detected a novel interaction between the extracellular domains of membrane proteins Islr2 and the Vasorin paralogs. Paired expression analysis suggested a functional role for these molecules in retinotectal axon guidance: *islr2* marks differentiated retinal ganglion cells, while Vasorin proteins are detected at the membrane along the complete retinal axon pathway. In particular, Vasnb marks a small set of midline glial cells at stages when the optic chiasm first forms. Contrary to rodents, zebrafish have completely crossed retinal axon projections. This makes the zebrafish an ideal model system to study default midline crossing decisions. We found that an *islr2* TILLING mutant displays an ectopic ipsilateral projection, alongside variable thinning of the optic nerve. We generated a *vasna* targeted mutant by TALEN mutagenesis and analyzed a *vasnb* TILLING mutant, but preliminarily detected no phenotype in these larvae. After inducing deletions in the *vasnb* locus by the CRISPR/Cas9 system in the *vasna* mutant background, we are in the process of analyzing double mutants for the two paralogs. In addition, we are attempting to describe the *islr2* guidance phenotype by live imaging of the retinal ganglion cell growth cones.

399. Characterization of the highly conserved m2de3 non-coding element associated with the *Meis2* gene. ***Alicia Ramsaran***[1], *Kyle Nelson*[2], *Ted Zerucha*[1]. 1) Appalachian State University, Boone, NC; 2) Wake Forest University Graduate School, Molecular and Cellular Biosciences, Winston-Salem, NC.
The Meis genes are known to play important roles in development. The Meis genes encode homeodomain proteins that are known to act as transcription factors and as cofactors of other homedomain proteins including members of the Hox and Pbx families. The purpose of this study is to gain a better understanding of the mechanisms that control the expression of the Meis genes during development. We have identified four highly conserved non-coding elements (NCEs) associated with the Meis2 gene. We have named these elements m2de1-4 (for Meis2 downstream element). All four of these elements have been identified in all tetrapods examined, however to date only one, m2de1, has been identified in teleosts. I am characterizing m2de3 to attempt to determine if it is able to direct spatial and temporal gene expression consistent with that of Meis2 using zebrafish as a model system. I have generated transgenic zebrafish with an expression cassette carrying the murine m2de3 element linked to a minimal promoter and the EGFP (enhanced green fluorescent protein) gene. In my experiments I observed punctate expression in the trunk of 48 hour zebrafish embryos. These results provide evidence that expression directed by m2de3 in spinal regions along the trunk is consistent with where Meis2 is expressed during mouse development.

400. Spatiotemporal Counteractions between The Defects of Spns1 and v-ATPase in Autolysosomal Regulation and Developmental Senescence. *Tomoyuki Sasaki*[1], *Shanshan Lian*[1], *Daniel Klionsky*[2], ***Shuji Kishi***[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) 2University of Michigan, Ann Arbor, MI.
Spinster homolog 1 (Spns1) in vertebrates, as well as Spinster (Spin) in *Drosophila*, is a hypothetical lysosomal H^+-carbohydrate transporter, which functions at a late stage of autophagy. The Spin/Spns1 defect induces aberrant autolysosome formation that leads to both embryonic senescence and accelerated aging symptoms in adults, while the molecular mechanisms of the pathogenesis still await elucidation. Using chemical and genetic approaches in zebrafish, we investigated a mechanism that ameliorates Spns1 loss-mediated senescence as well as autolysosomal impairment. We determined the effects of several chemical compounds and selective modulators of autophagy on Spns1 deficiency to see if any chemical(s) ameliorates or exacerbates the Spns1 phenotypes of zebrafish embryos. Of the

chemicals tested, bafilomycin A_1 and other proton pump inhibitors such as the FDA-approved acid reducer "omeprazole" stood out due to their apparent inhibitory effect on overall phenotypic deterioration in *spns1*-defective animals. We found that excessive lysosomal biogenesis and prolonged suboptimal acidification, modulated by the vacuolar-type H^+-ATPase (v-ATPase), could be the primary reason for the appearance of the hallmarks of Spns1 deficiency. Our findings thus suggest that Spns1 is critically involved in lysosomal acidification and trafficking during autophagy, and that spatiotemporal counteractions between the defects of Spns1 and v-ATPase may be essential for tipping the balance of autolysosomal regulation and developmental senescence.

401. Utility of Zebrafish Progeria Models in Dissecting Mechanisms of Aging. ***Chunmei Li***[1,2], *Christian Lawrence*[1], *Carrie Barton*[3], *Robert Tanguay*[3], *Matthew Harris*[1,2]. 1) Boston Children's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Oregon State University, Corvallis, OR.
Zebrafish is widely used to understand the genetic basis of vertebrate development and disease, but little attention has been paid to the mechanisms underlying aging and age-related pathologies. We asked if we could use the zebrafish as a tool to interrogate the timing of senescence and the molecular basis of aging. As an initial approach, we looked at mutants of known tissue senescence regulators, with a focus on the function of tert in regulating aging in zebrafish. Similar to recent findings from Henriques et al., (2013), we find phenotypes in tert mutant zebrafish comparable to those reported in mammalian mutants, including defects in proliferative tissues and reproduction. Furthermore, we investigated the regulation of tert-mediated senescence by performing sensitized screens in tert background. Although tert mutants have low fertility, we were able to isolate one unlinked dominant modifier that rescues reproductive capability. In addition, we wanted to test if we could isolate novel aging mutants using forward genetic screening. We isolated a progeric mutant, fruehrentner (frnt) that exhibits aging phenotypes as juveniles in a previous screen. We have mapped and are characterizing this mutant. The frnt progeric phenotype provides a means to perform efficient modifier screens for aging. In 1601 F1 progeny from mutagenized males crossed to frnt females, we found 10 non-complementing mutations in which one was unlinked to the frnt locus. Thus, through a small-scale screen we were able to identify novel loci regulating the function of frnt. The identification of suppressors shows potential of identifying longevity genes - which could not efficiently be done with normally aged wild type fish. The aging models in zebrafish permit exploration of the molecular and physiological regulation of aging. To this end, we have defined a caloric restriction model in zebrafish that is able to rescue the decreased longevity in frnt. The presented zebrafish models of aging broaden our knowledge of the genetic control of aging and provide tools to assess treatment of aging-associated pathologies.

402. FGF1 Mediates Overnutrition-Induced b Cell Differentiation. ***Mingyu Li***, *Wenbiao Chen*. Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.
Increased insulin demand such as insulin resistance and overnutrition induces expansion of b-cell mass but the underlying mechanism is largely unknown. We have previous reported that overnutrition rapidly induces b-cell differentiation through prolonged stimulation of existing b cells in zebrafish larvae (Maddison and Chen. Diabetes, 2012; Li et.al. Am J Physiol Endocrinol Metab, 2014). These data suggest that b cells are the sensor of overnutrition and hyperactivated b cells emit a signal to induce the differentiation of endocrine precursor cells. To identity the b-cell derived neogenic signals, we examined the role of several signaling pathways through pharmacological manipulations. We found that FGF signaling is necessary for the overnutrition-induced b-cell differentiation as FGFR inhibitor SU5402 suppressed the differentiation. Furthermore, we discovered that the non-canonically secreted FGF1 is the likely FGFR agonist since neocuperione, a Cu^{2+} chelator that inhibits FGF1 release, also significantly blunted the differentiation. To validate the role of FGF1, we generated *fgf1* mutant alleles using TALEN mutagenesis. We found that the overnutrition-induced b-cell differentiation is abolished in *fgf1*$^{-/-}$ larvae. Conversely, targeted expression of a human FGF1 with an engineered signal peptide in b cells increased b-cell number. Our data suggest that overnutrition may promote FGF1 secretion from hyperactivated b cells, which in turn induces endocrine precursor cell to differentiate into b cells.

403. Transcriptional regulation of cold acclimation in zebrafish. ***Y. Long***, *Z. Cui.* Institute of Hydrobiology, CAS, Wuhan, China.
Environmental temperature variations affect many properties and functions of biomolecules and structural components of the cell, such as folding, assembly, activity and stability of proteins, structure and rigidity of lipids, and fluidity and permeability of cell membrane. Body temperature of most fishes equilibrates rapidly with ambient temperature, so water temperature is the abiotic master factor that virtually controls and limits all the biochemical, physiological and life history activities. Temperature is also regarded as a lethal factor for fishes and most of fish kills in nature are caused by exposure to low temperatures. To combat the adverse effects elicited by chilling and maintain cellular functions at reduced temperatures, fishes have evolved versatile mechanisms that enable them to acclimate cold stresses. Transcriptional regulation is the critical step for acclimation processes of organisms under various stresses. Our studies demonstrated that pre-exposure of zebrafish larvae at 96 hpf to mild cold stress (16 oC) for 24 h significantly increased their resistance to severe cold stress at 12 oC. Microarray analysis has identified multiple genes regulated by cold stress in zebrafish larvae exposed to 16 oC for 2 h or 48 h, especially transcription factors up-regulated at 2 h after cold exposure. RNA-seq studies in zebrafish larvae exposed to 16 oC for 24 h have disclosed alternative promoter usage and alternative splicing events specifically regulated by cold stress. Gene ontology enrichment analysis of cold-induced genes has revealed that RNA splicing, ribosome biogenesis and protein catabolic process are the most highly overrepresented biological processes. Spliceosome, proteasome, eukaryotic ribosome biogenesis and RNA transport are the most highly enriched pathways for genes up-regulated by cold stress. Our results indicate that zebrafish larvae possess the ability to build cold-tolerance under mild low temperature and transcriptional and post-transcriptional regulations are extensively involved in this acclimation process. These findings would be very important for further understanding the intracellular signaling mechanisms of cold stress in fish.

404. Cystic Fibrosis of the Zebrafish Pancreas. ***Adam Navis***, *Michel Bagnat.* Department of Cell Biology, Duke University Medical Center, Durham, NC.
Cystic Fibrosis (CF) is a multi-organ disease caused by loss of activity of a chloride channel, CFTR, which leads to reduced luminal fluid secretion and mucosal buildup, blocking function of many organs. In the pancreas, ductal blockage is thought to drive destruction of the exocrine pancreas, causing pancreatic insufficiency in a large number of patients. Several animal models have been generated to characterize the pathophysiology of CF; however, they lack the developmental and genetic tools to examine the early stages of pancreatic disease. In adult *cftr* mutant zebrafish, we observe widespread destruction of the exocrine pancreas and cystic fibrosis in a pattern similar to human disease. The zebrafish *cftr* mutants have other hallmarks of CF, including reduced growth rates and male infertility, indicating that the zebrafish is a good model for CF. We observed *cftr* expression within the pancreatic duct throughout morphogenesis. *In situ* hybridization and expression of a bacterial artificial chromosome (BAC) transgene encoding a Cftr-GFP fusion protein indicate that *cftr* is expressed in the pancreatic duct as early as 3 days post fertilization (dpf) and continues to be apically localized within the duct in the adult pancreas, consistent with mammalian CFTR expression and localization. During the initial stages of pancreatic development, specification of exocrine, endocrine, and ductal cells occurs normally. However, in *cftr* mutants, reduced growth rates and pancreatic destruction are apparent by 21 dpf. Here we characterize the onset of pancreatic disease in a zebrafish model for CF using transgenic markers of the exocrine pancreas, inflammatory responses, and immune cells. The zebrafish represents an exciting new model for understanding the pathophysiology of CF and the development of new therapeutic targets for the disease.

405. The study of inflammatory response in a zebrafish model of epilepsy. ***Valeria Nittoli***[1,2], *P. De Girolamo*[2], *P. Sordino*[1,3]. 1) Laboratory of Cellular and Developmental Biology, Stazione Zoologica Anton Dohrn, Naples, Italy; 2) Department of Veterinary Medicine and Animal Productions, University of Naples 'Federico II', Naples, Italy; 3) Institute for Mediterranean and Forestal Systems (ISAFOM-CNR), Catania, Italy.
In experimental and clinical models of epilepsy, brain inflammation has the potential to modulate seizure threshold and recurrence, to regulate cell survival and rewiring of neuronal circuits, leading to the establishment of hyperexcitable neuronal networks. Astrocytes appear to be the major source of pro-inflammatory molecules associated to the epileptogenic process, with the involvement of specific inflammatory pathways (such as IL-1b/toll-like receptors). However, understanding the immunological mechanisms underlying epilepsy and the effective therapeutic treatments remains incomplete. Zebrafish is a prominent vertebrate model for studying epilepsy mechanisms and for anti-epileptic drug discovery. Here, we present a molecular and cellular characterization of the inflammatory response in the immature brain of a zebrafish Pentylenetetrazole (PTZ, a chemical convulsant) seizure model, including a focus on seizure-induced neuronal injury. Wt and tg(gfap:GFP) zebrafish larvae at 7 dpf are treated with PTZ for different durations and then processed for molecular (IL-1b, TLR) and cellular (TUNEL, HuC, PCNA, GS, GFAP) markers. Then, we explore the role of IL-1b by drug treatment and knockdown approach. Moreover, we examine astrocytes activation in the immature epileptic brain by focusing on the telencephalic area. Finally, we search for signs of neuronal loss/regeneration and analyze how these processes affect the normal brain circuits. Our results suggest that the inflammatory response in the zebrafish PTZ seizure model recapitulates the clinical manifestation observed in human and rodent brain. In addition, PTZ treatment reveals IL-1b upregulation and astrocyte activation (both fibers and cell number) in a time-dependent and exponential way. These observations, in our opinion, add an important value to the use of zebrafish model in the study of epilepsy, giving the opportunity to dissect the inflammatory components and mechanisms involved in the epileptic state.

406. Dual mechanisms combine to mediate regeneration of melanocytes following injury. ***Sharanya Iyengar***, *Craig Ceol.* Program in Molecular Medicine and Department of Cancer Biology, UMass Medical School, Worcester, MA.
Melanocytes are pigment-producing cells in the skin of vertebrates that can be lost during hair graying, injury and disease-related depigmentation. Melanocytes are replenished in mammals by resident stem cells. To gain insight into melanocyte regeneration, which is poorly understood, we set out to identify whether melanocyte stem cells are present in adult zebrafish and how such cells might reconstitute the pigment pattern following injury. Using a targeted cell ablation approach we determined that *mitfa* is expressed not only in differentiated melanocytes but also in the cells that mediate melanocyte regeneration. When *mitfa*-positive cells are selectively ablated no melanocyte regeneration occurs. However, when ablation is performed in a *p53*-deficient background, melanocyte regeneration occurs, suggesting that death of the cells that mediate regeneration is dependent on *p53*. We then used *mitfa*-positivity to perform lineage-tracing experiments and assay whether unpigmented *mitfa*-expressing cells have stem cell properties. During regeneration, *mitfa*-positive cells can divide asymmetrically with one daughter cell differentiating and the other daughter remaining uncommitted; these are melanocyte stem cell divisions. In addition, some *mitfa*-positive cells directly differentiate during regeneration. Taken together, these data indicate that multiple mechanisms are used to re-establish pigmentation following injury and enable regeneration following subsequent rounds of ablation. We have used reporter assays and drug studies to assess whether pathways important for melanocyte development are also involved in regeneration. We found that Wnt signaling gets turned on during melanocyte regeneration and that Wnt inhibition after ablation of differentiated melanocytes delays regeneration. These studies have established a system by which regeneration can be traced with single-cell resolution and perturbations to regeneration analyzed in exquisite detail.

407. Analysis of Fibroblast Growth Factor Signaling in the Regenerating Zebrafish Kidney. ***Jonathan Jou***, *Robert McKee, Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
Kidney damage begins at the nephrons, which are specialized epithelial tubules that constitute the functional units of the kidney. The nephrons filter the blood, acting as an important component of removing waste and nutrients from the body to maintain homeostasis. Kidney dysfunction results when nephron cells are injured and destroyed, which can lead to acute or chronic renal disease. Research toward the development of regeneration-based treatments utilizes zebrafish as a model organism because of its robust regenerative capacities and high degree of conservation between the fish and mammalian nephron structures. Kidney regeneration in the adult zebrafish has been

recently shown to entail epithelial regeneration of damaged nephrons and development of entirely new nephrons from renal progenitors, a phenomenon termed neonephrogenesis. The molecular basis for both these regeneration responses remains unknown. Fibroblast growth factor (Fgf) signaling plays diverse roles in development and regeneration of tissues like the heart and fin, and thus is an attractive candidate pathway to evaluate with regard to the kidney. Thus, we surveyed the expression of a panel of Fgf ligands and receptors by whole mount *in situ* hybridization and RT-PCR in zebrafish kidneys at 0, 1, 3, 5, 7, and 10 days post injury from the aminoglycoside antibiotic gentamicin, which has been shown to preferentially damage the nephron proximal tubule. These approaches identified discrete spatiotemporal expression of the receptor *fibroblast growth factor receptor (fgfr) 1a* and the ligand *fgf8a* in uninjured and regenerating kidneys. Quantitative real time polymerase chain reaction assays showed an increased expression of both *fgf8a* and *fgfr1a* mRNA at the established time points of regeneration. Further, mRNA transcripts were co-localized between *fgfr1a* and transcription factors associated with neonephron structures, namely *wt1b* and *lhx1a*. Taken together, these data suggest that Fgf signaling involving Fgf8a and Fgfr1a may play roles in the regeneration response. Future experiments will use chemical genetics and transgenics to perform gain and loss of function studies to evaluate the outcome of alterations in Fgf signaling during regeneration.

408. Wnt signaling is required for zebrafish kidney regeneration. ***Caramai N. Kamei***[1,2], *Yan Liu*[1,2], *Neil A. Hukriede*[3], *Iain A. Drummond*[1,2]. 1) Nephrology Division, Massachusetts General Hospital, Charlestown, MA; 2) Dept. of Genetics, Harvard Medical School, Boston, MA; 3) University of Pittsburgh, Pittsburgh, PA.
Kidney regeneration in zebrafish occurs by repair of existing nephrons as well as by *de novo* generation of new nephrons from adult organ progenitor cells by neonephrogenesis. We report here that Wnt signaling plays an essential role in adult zebrafish kidney regeneration. Newly forming nephron condensates in the developing mesonephros as well as those formed in the adult in response to gentamicin injury express the Wnt receptor *frizzled9b* (*fzd9b*) and the canonical Wnt target gene *lef1*. In the Tg(*lhx1a:gfp*) nephron progenitor reporter line, both individual lhx1a-positive adult kidney progenitor cells and nephron condensates are specifically marked by expression of *fzd9b*. Pharmacological blockade of Wnt signaling using IWR1 and IWP2 led to a decrease in *lhx1a+* nephron condensates after injury. EdU labeling in Tg(*lhx1a:gfp*) fish after gentamicin injury reveals that newly forming condensates are actively proliferating and this proliferation is blocked by Wnt inhibition. Our results demonstrate an essential role for Wnt signaling in adult zebrafish kidney regeneration. Identification of *fzd9b* as a new marker of adult kidney progenitor cells opens new avenues to investigate their developmental origins and regenerative potential.

409. Genetic analysis of male sex ornament maintenance/homeostasis and relationship to fin regeneration. ***Junsu Kang***, *Kenneth Poss.* Cell biology, Duke University, Durham, NC.
Precise regulation of signaling activators and inhibitors can help limit developmental crosstalk between neighboring tissues during morphogenesis, homeostasis, and regeneration. Here, we find that the secreted Wnt inhibitor Dkk1b is abundantly produced by dense regions of androgen-regulated breeding tubercles (BT) on the surfaces of adult male zebrafish pectoral fins. High-speed videos and amputation experiments reveal that pectoral fins and their BT are employed for male spawning. Formation and rapid turnover of BT involve Dkk1b induction and maintenance, whereas Dkk1b is typically restricted from the regeneration blastema after amputation injury. When amputation occurs through a BT-containing region, a Dkk1b-enriched wound epidermis forms and blastema formation is disrupted, compromising regeneration. Our experiments illustrate how homeostatic signaling by key breeding ornaments can interfere with injury-activated tissue regeneration. These findings help explain sexually dimorphic fin regeneration in zebrafish, and have implications for how regenerative potential might decline as development progresses or during species evolution. To identify further cellular and molecular mechanisms for homeostasis of male breeding ornaments, we are currently performing a forward genetic screen for BT-defective mutants.

410. Regeneration of fin in zebrafish, Danio rerio. ***Pancharatna A. Katti***, *Basavaraj Goundadkar, Narshimamurthy Anegundi.* Dept of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India.
Regeneration is a phenomenon of reactivation of development in postembryonic life to restore and replace lost tissue that involves cell proliferation, differentiation, growth and morphogenesis. Although teleost fishes are known to regenerate multiple structures, experimental data to support this statement are meager. The present study is an attempt to elucidate the regenerative abilities of pectoral, pelvic, dorsal and anal fins of zebrafish using amputation technique Adult (body size: 3.5 ± 0.5 cm) zebrafish (wild type) procured from local vendors were maintained in the laboratory under natural temperature (26 ± 1º C) and photo phase (11.30 -12.30 hrs) in well aerated glass aquaria with conditioned water for a week prior to experimentation. Six fish were used for each (pelvic, pectoral, dorsal and anal) fin amputation and six fish were maintained as controls (without amputation with intact fin). Amputations were performed under cold anesthesia using a sharp blade. One fin amputation was performed for each fish. Two experiments were conducted. In experiment one, after amputation, a follow up observation of the fin regeneration was performed and regenerated portion of the fin was measured and photographed on every third day for thirty days and compared with corresponding controls. In the second experiment repeated amputations of the same fin in a single fish were performed once in a month for three consecutive months in order to know whether the regenerative ability is multiple or ceases with a single amputation. Our observations suggest that all the fins of zebrafish undergo regeneration and have the ability to restore the lost part of the fin. However, the regenerative ability gradually decreases with repeated amputations. Of all the fins pectoral fin has the greater ability for regeneration.

411. Control of Inflammatory Responses during Heart Regeneration in Zebrafish. *Delicia-Zhe Sheng, Alvaro Gonzalez-Rajal,* ***Kazu Kikuchi***. Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010 Australia.
Unlike mammals, certain vertebrates such as urodele amphibians and teleost fish possess a remarkable capacity for cardiac regeneration in adult, providing a model to understand how regeneration might be induced in the human heart. Among these models, the zebrafish offers substantial advantages in the use of genetic techniques such as mutagenesis, transgenesis, as well as gene targeting, and has been

established as a standard model for studying natural mechanisms of heart regeneration. Previous studies demonstrated that cardiac muscle in the zebrafish heart regenerates largely through dedifferentiation and proliferation of existing cardiomyocytes, and signals derived from epicardial and endocardial cells promote the cardiomyocyte proliferation after injury. However, contributions and roles of non-cardiac cells during zebrafish heart regeneration remain unclear. Using transgenic reporter strains and gene expression analyses, we have been characterizing the cell types and responses of immune cells in the regenerating zebrafish heart, and have found a novel cell population that may provide a key contribution to modulating inflammatory events induced by injury. The functional characterization of this cell type and its possible role on heart regeneration will be discussed.

412. Characterizing the role of Lgr6 in hair cell progenitors of the lateral line neuromast. ***Jonathan Kniss***, *Tatjana Piotrowski.* Stowers Institute for Medical Research, Kansas City, MO.
Mechanosensory hair cells in the zebrafish, and other nonmammalian vertebrates, are capable of regeneration after damage, whereas mammals are largely unable to regenerate hair cells. However, across vertebrates many of the same pathways are required for hair cell differentiation: Wnt/b-Catenin signaling promotes proliferation of sensory progenitor cells and Fgf and Notch signaling are required to activate and restrict expression of the prosensory transcription factor atoh1, that is also required for differentiation and hair cell maturation. The zebrafish lateral line is composed of mechanosensory organs, or neuromasts, that are functionally analogous to the mechanoreceptors of the mammalian inner ear. Mature hairs cells within each neuromast are constantly replenished during homeostasis, and hair cell regeneration occurs rapidly after damage. Therefore, the zebrafish lateral line is a powerful system to examine complex signaling interactions during hair cell differentiation and regeneration. The Lgr family protein Lgr6 is expressed in support cells that surround lateral line hair cells during development, homeostasis and regeneration. Lgr6 is a zebrafish orthologue of the mammalian Lgr5, which is a Wnt/b-Catenin target and serves as a stem cell marker in the intestinal epithelium and hair follicle niches. Lgr5-expressing support cells in the mammalian cochlea are hair cell progenitors that are activated by Wnt/b-Catenin signaling. Similarly, we find that Lgr6 expression in the lateral line is modulated by attenuation or activation of Wnt/b-Catenin signaling. This observation, when combined with Lgr6 expression in support cells, suggests *lgr6* may be a marker of hair cell progenitors as in the mammalian cochlea. In further support of this, we find that *lgr6* expression is downstream of both Notch and Fgf signaling, as might be expected if *lgr6* expressing cells are specified as hair cell progenitors. The relationships between Lgr6 expression and the Wnt/b-Catenin, Fgf and Notch signaling pathways are also maintained in mature sensory organs. We are currently testing if *lgr6* also plays a role during hair cell regeneration.

413. The role of the actin cytoskeleton and Rho-associated coiled-coil kinases in the adult regenerating zebrafish retina. ***Manuela Lahne***, *David R Hyde.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
In the adult zebrafish retina, dying photoreceptors induce Müller glia to divide and produce neuronal progenitor cells (NPCs) that subsequently differentiate and replace lost photoreceptors. Similar to NPCs undergoing interkinetic nuclear migration (INM) during retinal development, proliferating Müller glia nuclei migrate between the basal and apical limits of the retina in phase with the cell cycle during regeneration. Actin-myosin mediated contraction facilitates INM during retinal development. To test whether a similar mechanism acts during retinal regeneration, light-damaged *Tg[gfap:EGFP]* transgenic zebrafish that express EGFP in Müller glia, were exposed to CytochalasinD or Rockout, an actin polymerization or Rho-associated coiled-coil kinase (Rock) inhibitor, respectively. Immunocytochemical labeling of the mitosis marker phospho-histone 3 (pH3) was used to assess the position of dividing Müller glia nuclei in drug exposed retinal sections. At 35 hours of light-treatment, pH3-positive Müller glial nuclei in DMSO controls had predominantly migrated apically within the inner nuclear layer (INL) or into the outer nuclear layer (ONL). In contrast, both CytochalasinD and Rockout caused significant increases in the number of pH3-positive Müller glial nuclei in the basal and apical INL at the expense of those in the ONL. Disrupting INM by inhibiting Rock significantly reduced the number of proliferating cells at 45 and 72 hours of light-treatment. EdU and BrdU pulse-chase experiments which labeled activated Müller glia and dividing NPCs, respectively, revealed fewer EdU- and BrdU-double-positive cells at 45 and 50 hours of light-treatment in Rockout-exposed retinas, suggesting premature cell cycle exit. Moreover, significantly fewer blue and red cones were regenerated at 8 days of recovery (post light-damage) in Rock-inhibited retinas relative to DMSO controls. In conclusion, disrupting the actin cytoskeleton interferes with INM, NPC proliferation and photoreceptor regeneration.

414. Differential roles of Mmp9 in acute and chronic inflammation. ***D. LeBert***[1,2], *J. Rindy*[2], *J. Squirrell*[3], *A. Zakrzewska*[4], *A. Meijer*[5], *A. Huttenlocher*[2,6]. 1) Cell and Molecular Pathology, University of Wisconsin, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI; 4) Scientific Advisor Vaccine Discovery at Crucell Amsterdam Area, Netherlands; 5) Institute of Biology, Leiden University, Leiden, The Netherlands; 6) Department of Pediatrics, University of Wisconsin-Madison, Madison, WI.
Chronic inflammation is associated with human disease, including psoriasis. We previously described a zebrafish chronic inflammation mutant, induced by an insertion in the Hepatocyte growth factor activator inhibitor 1 gene (hai1; also known as Spint1), which showed epidermal hyperplasia and chronic epidermal inflammation, similar to human psoriasis. Here, using gene expression analysis (microarray), we identified genes upregulated in two different models of zebrafish chronic inflammation. One of the highly up-regulated genes in hai1 mutants was matrix metalloproteinase-9 (Mmp9). To determine if Mmp9 mediates the chronic inflammation and epidermal phenotype we depleted Mmp9 using MO. Depletion of Mmp9 partially rescued the epidermal phenotype. Additionally, mmp9 expression increased in epidermal cells acutely after wounding, as detected by tissue specific translating ribosomal affinity purification (TRAP). Depletion of Mmp9 impaired wound resolution and regeneration after tail transection, suggesting that Mmp9 has different roles in acute and chronic tissue damage. To further understand the defect in wound resolution we performed second harmonic imaging (SHG) to visualize collagen organization in both hai1 and Mmp9 depletion models. SHG revealed reduced fiber widths and alterations in collagen alignment in the regenerating fins of Mmp9 deficient embryos and collagen organization defects in the hai1 mutants. Taken together, Mmp9 mediates

inflammation in acute and chronic wounding, but is necessary for wound resolution after acute damage, likely in part through the re-organization of the extracellular matrix.

415. Characterization of the roles for ADAM17b in the adult zebrafish retinal regeneration. ***Jingling Li****, Josh Hobgood, David.R Hyde.* Biological Science, University of Notre Dame, Notre Dame, IN.
The zebrafish retina possesses the ability to restore any neuronal types that are lost upon injury. In response to retinal cell loss, zebrafish Müller glial cells dedifferentiate and re-enter the cell cycle to produce neuronal progenitor cells that continue to proliferate and differentiate into any retinal neuronal type that was lost. Previous studies demonstrated that several genes and signaling pathways are involved in retinal regeneration, including TNFa and Notch. TNFa is a damage signal that produced by apoptotic photoreceptors that is necessary and sufficient to stimulate Müller glial cell dedifferentiation and proliferation. In contrast, Notch signaling represses Müller glia dedifferentiation and proliferation, while facilitating cell differentiation . Interestingly, both TNFa and Notch must be processed through cleavage events to be activated and A Disintegrin And Metalloproteases (ADAM) protein family members are likely candidates for this protease. This project aims to study the potential roles of ADAM proteins in TNFa and Notch processing in Müller glia dedifferentiation and proliferation. As a starting point, I examined the two zebrafish ADAM17 paralogs, ADAM17a, and ADAM17b. We found that morpholino-mediated knockdown of either ADMA17a or ADAM17b expression resulted in a significant reduction in the number of proliferating Müller glial cells, which suggested that both ADAM17a and ADAM17b are required, and might serve different functions, during retinal regeneration. Furthermore, ADAM17b was necessary for cone, but not rod photoreceptor regeneration following light-induced retinal damage. Intravitreal injection of TNFa into adam17b morphant eyes rescued Müller glial proliferation, suggesting that ADAM17b is required for TNFa cleavage and activation. Blocking Notch signaling by injecting the g-secretase inhibitor RO4929097 in adam17b morphant eyes also induced Müller glial proliferation, but without proper redial migration. This suggests that ADAM17b does not cleave the Notch receptor involved in Müller glia quiescence. Future studies will examine the potential roles of ADAM17a in the proteolytic processing of TNFa and Notch in retinal regeneration.

416. Characterization of the zebrafish *godot* mutant, which fails to regenerate lateral line sensory hair cells. ***Mark E Lush***[1]*, Megan Smith*[2]*, Tatjana Piotrowski*[1,2]*.* 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Utah, Department of Neurobiology and Anatomy, Salt Lake City, UT.
The mechanosensory lateral line of zebrafish consists of neuromasts that contain sensory hair cells, support cells and stem cells. The sensory hair cells of the lateral line are analogous to sensory hair cells in the inner ear. Importantly, zebrafish hair cells are able to regenerate after damage unlike their mammalian counterparts. Hair cell regeneration occurs through proliferation and differentiation of a stem cell pool, which can give rise to both support cells and hair cells. The genes and signaling pathways required for zebrafish hair cell regeneration are currently unknown. Our laboratory is performing an ENU based forward genetic screen to identify novel genes required for hair cell regeneration. Here we describe the characterization of one of these mutants, *godot*. Early lateral line development is normal in *godot*, but mutant larvae fail to regenerate appropriate hair cell numbers after neomycin-induced hair cell death. After hair cell death *godot* neuromasts express similar levels of *sox2* and *atoh1a* when compared to controls, but show reduced expression of the differentiating markers *pou4f3* and *gfi1*. Surprisingly, support cell proliferation is increased in *godot* larvae compared to controls after neomycin treatment. This increased proliferation could be due to a failure of hair cell precursors to exit the cell cycle or newly formed hair cells do not survive and trigger renewed support cell proliferation. Additionally, we observed a decreased immune response to damaged neuromasts in *godot*. Wither this decreased immune response is causative, or a result, of the failure to regenerate is currently unclear. Whole genome DNAseq has mapped the mutation to a chromosomal location and appears to be within a non-coding region that we are currently investigating in more detail. This unbiased approach to identify genes required for sensory hair cell regeneration in zebrafish will lead to a better understanding of the signaling pathways involved in regeneration and guide the development of potential therapeutic targets for people.

417. Hyaluronic Acid and its receptor *hmmr* promote epicardial cell migration and angiogenesis during zebrafish heart regeneration. ***M. A. Missinato***[1,2]*, K. Tobita*[1]*, N. Romano*[2]*, J. A. Carroll*[3]*, M. Tsang*[1]*.* 1) Developmental Biology, University of Pittsburgh, Pittsburgh, PA; 2) Ecology and Biology, University of Tuscia, Viterbo, Italy; 3) Rocky Mountain Laboratories, Laboratory of Persisten Viral Diseases, Hamilton, MT.
Zebrafish can naturally regenerate the heart after ventricle amputation. From a proteomic study, we identified Hyaluronan mediated motility receptor (Hmmr), a hyaluronic acid (HA) receptor, to be upregulated at 3 days post amputation (dpa). HA is a component of the extracellular matrix (ECM) that binds to the receptors CD44 and Hmmr to modulate cell migration, proliferation, and cellular signaling in wound healing and tissue repair. qPCR confirmed that *hmmr* and *cd44* transcripts as well as enzymes involved in HA synthesis, hyaluronic synthases 1 and 2 (*has1* and *has2*), were upregulated following cardiac injury. Using a biotinylated HA-binding protein (bHABP), we detected an increase of HA accumulation in regenerating hearts that was localized in the clot area at 3 dpa, suggesting a potential role for HA in cardiac regeneration. Indeed, chemical inhibition of HAS by retroorbital injections of 7-Hydroxy-4-methylcoumarin (HMC), a small molecule that specifically blocks HAS function, suppressed cardiac regeneration implicating a critical role for HA in this process. HMC injection blocked epicardial cells migration in the regenerating area and suppressed coronary vasculature formation, a process that is important to support new cardiac muscle. Cell migration was blocked because HMC inhibited the phosphorylation of focal adhesion kinase (FAK), a protein that promotes cell contacts with the ECM, allowing cell migration. Additionally, studies using vivo morpholinos targeting *hmmr* suppressed heart regeneration, confirming that HA and its receptor are critical for zebrafish heart regeneration. The rapid response in HA production after cardiac injury led us to determine if a similar mechanism exists in mammals following ischemic damage. In a rat model of myocardial infarction both HA and HMMR were upregulated and localized in the infarct area within the first few days following damage suggesting that this pathway may also play an important role in cardiac repair in mammals.

418. Genome-wide and organ-specific landscape of long non-coding RNAs in zebrafish. *Kriti Kaushik, Elvin Leonard Vincent, Shamsudheen KV, Ambily Sivadas, Saakshi Jalali, Shadabul Haque, Vinod Scaria,* ***Sridhar Sivasubbu***. CSIR Institute of Genomics and Integrative Biology, Delhi, Delhi, India.
Long non coding RNAs (lncRNAs) are unannotated class of transcripts that are more than 200nt in length and do not have the potential to code for functional peptides. Studies have shown that lncRNAs can regulate gene expression by diverse mechanisms. The spatio-temporal expression dynamics of lncRNAs has been documented in embryonic zebrafish. However, their expression as well as role in the maintenance of form and function in adult zebrafish tissues has not been fully explored. Therefore, to catalogue the lncRNAs in specific tissues of zebrafish we performed polyA RNA sequencing on RNA isolated from five different organs followed by analysis through a stringent computational pipeline to enlist lncRNAs expressed in adult zebrafish. Our study reports 419 novel lncRNAs out of the 442 predicted lncRNA transcripts. Tissue restricted expression was observed for 77 transcripts across the five major organs investigated, with brain (47) having the maximum number of lncRNAs followed by heart (12), blood (12), muscle (4) and liver (2). The expression of these in-silico predicted lncRNAs was further validated quantitatively and qualitatively through real time PCR and in-situ hybridization. Our study provides a baseline data for understanding the molecular interaction in gene expression within the specific adult tissues of zebrafish.

419. Profiling of the Transcriptome During Oogenesis in the Zebrafish and a Novel Oocyte Specific RNA Species. ***Caitlin Stewart***[1]*, S. Harvey*[1]*, J. Zamora*[1]*, I. Sealy*[1]*, F. Marlow*[2]*, D. Stemple*[1]. 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Albert Einstein College of Medicine, NY, US.
The growth and development of an oocyte into an egg involves changes in gene expression and regulation as well as unique RNA processing mechanisms important for later embryonic development. During oogenesis, a unique silent chromatin landscape is established, allowing initial embryonic development to be directed solely by these uniquely processed RNA transcripts. The transcriptional processes that underlie these mechanisms have yet to be described. We aim to characterise and compare the global transcriptional profile of each stage of oogenesis using RNA-seq technology and to functionally validate interesting genes using CRISPRs and ENU mutants available from the Zebrafish Mutation Project. This will provide a greater understanding of the biology of oogenesis and increase our understanding of early vertebrate development. During preliminary transcriptome analysis, a novel oocyte-specific RNA species was observed in stage I oocytes, the characterisation of which has become the centre of our investigations.

420. Loss of Crb2b^{LF} Leads to Enlargement of Cone Outer Segments in the Zebrafish Retina. *Marta Luz,* ***Satu Kujawski****, Cátia Crespo, Monalisa Mishra, Elisabeth Knust.* Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
Crb proteins are apical determinants of apico-basal epithelial polarity. In *Drosophila*, the prototypic Crb has been shown to regulate morphogenesis and maintenance of the highly polarized photoreceptor cells (PRC). In zebrafish, *crb1*, *crb2a*, and *crb2b* have previously been reported to be expressed in the retina; Crb2a and Crb2b are known to control the development of polarity in the retinal primordium as well as the integrity of the PRC layer (Omori et al. 2006; Zou et al. 2012). Furthermore, *CRB1* mutations in humans cause retinitis pigmentosa and Leber's congenital amaurosis. However, the mechanisms mediating PRC degeneration upon loss of CRB1 are not understood. Using TILLING, we identified a mutation in the zebrafish *crb2b* gene leading to an early stop codon in the first EGF-like repeat of the protein. This mutation specifically affects the previously reported Crb2b long form (LF, Zou et al. 2012). To characterize the expression pattern of wild type Crb2b in the eye, we raised an antibody that recognizes both the long and short isoforms of Crb2b. By immunostaining of larval retinal sections, we show that Crb2b is most strongly expressed in the newly differentiated PRCs. This peripherally predominant expression persists in the adult retina. *crb2b*^{LF} mutants are homozygous viable, fertile and have no gross morphological defects. In the retina, lamination is intact. However, a detailed analysis of the retina revealed that *crb2b*$^{LF-/-}$ cones have enlarged outer segments (OS). This difference in OS size can already be detected at 3 dpf. The defect is cell autonomous, since *crb2b*$^{LF-/-}$ cells in an otherwise wild type retina also display this phenotype. Inner segment size is unaffected by this mutation. Our results demonstrate a role for Crb2b^{LF} in the regulation of cone OS size. Previously, overexpression of Crb2a or loss of Moe, a negative regulator of Crb, has been shown to influence the size of the PRC OS (Hsu et al. 2006; Hsu & Jensen, 2010). Together, these data suggest that maintaining a tight regulation of Crb levels is critical for PRC morphogenesis. Moreover, as the *crb2b*^{LF} mutants are viable, this line provides an important tool for the study of PRC OS development and maintenance.

421. Hedgehog signaling controls cell movements underlying choroid fissure formation. *Emily O. Wirick,* ***Kristen M. Kwan***. University of Utah, Salt Lake City, UT.
Optic cup morphogenesis establishes the basic structure of the vertebrate eye. In zebrafish, this process occurs rapidly (12-24 hpf): the optic vesicle evaginates from the brain, and undergoes cell and tissue rearrangements to generate the optic cup, with neural retina and retinal pigmented epithelium surrounding the lens. During this process, the choroid fissure also forms: this critical yet transient structure at the ventral eye creates the channel through which retinal vasculature enters the eye and retinal ganglion cell axons exit. Failure of choroid fissure development results in coloboma, a birth defect associated with visual impairment. Human genetic studies have identified numerous genes which, when mutated, result in coloboma. Notably, mutations that result in overactive Hedgehog signaling lead to coloboma, yet how this occurs remains unknown. Our goal is to determine the cellular and molecular mechanisms by which overactive Hedgehog signaling results in coloboma. Though much work has focused on its role in patterning the optic vesicle, we hypothesized that Hedgehog signaling might also control cell movements underlying choroid fissure formation. Using a combination of 4-dimensional timelapse imaging, cell tracking, and the photoactivatable fluorophore Kaede, we have, for the first time, determined the origin of and cell movements underlying normal choroid fissure formation, as well as how they are disrupted when Hedgehog signaling is overactivated, either by mutation of the *patched2* gene (*blowout*^{tc294z}) or expression of an activated form of Smoothened. We find that overactive Hedgehog signaling specifically disrupts very early cell movements underlying choroid fissure formation: cells destined for the choroid fissure are inhibited from migrating into the optic vesicle during evagination, while movements of other cells populations are unaffected. As a result, the optic cup develops

with a malformed choroid fissure. Disruption of the cell movements underlying choroid fissure formation represents a novel mechanism underlying this birth defect. We are currently working to determine whether Hedgehog signaling is acting canonically or non-canonically to control cell movements.

422. Wnt and FGF signaling coordinate posterior lateral line neuromast differentiation following deposition. *Danielle Bynoe, Parker Lewis, Jeffery Head, **Jason Meyers**.* Biology, Colgate University, Hamilton, NY.
Wnt and FGF signaling control the proliferative growth and migration of the posterior lateral line primordium, as well as the deposition and organization of protoneuromasts from the migrating primordium. Although these two signals are well established in these early stages of lateral line development, less is known about the signaling that coordinates proliferation of progenitor cells within the protoneuromasts following their deposition, or that controls the differentiation of the cells into a mature neuromast. We have previously shown that Wnt signaling is important in controlling proliferation of cells in the neuromasts after their deposition, during initial differentiation, ongoing-growth, and regeneration following lesion. Given the interaction between Wnt and FGF signaling in initial patterning of the primordium and protoneuromast deposition, we have examined whether FGF signaling interacts with the Wnt pathway to coordinate later stages of neuromast development, growth and regeneration. Inhibition of FGF signaling following deposition of the neuromasts does not alter the number of hair cells that are produced but has a slight effect on morphogenesis of the neuromasts. However, simultaneous activation of Wnt signaling and inhibition of FGF signaling increases proliferation, significantly limits the number of hair cells that differentiate, and leads to a dramatic disruption of protoneuromast structure after deposition. In particular, the cells in the deposited protoneuromasts lose N-cadherin expression, take on a mesenchymal-like morphology, and return to migratory behavior, moving bidirectionally along the lateral line. The cells retain their lateral line identity, and reconstruct neuromasts with differentiated hair cells following washout of the drugs. We also find that these two signals also have synergistic effects during regeneration following hair cell loss. These data suggest that Wnt and FGF signaling work together not just during initial deposition of protoneuromasts, but also in later stages of neuromast differentiation to maintain the epithelial organization of the neuromast and the balance between proliferation and differentiation.

423. Ethanol-induced Defects in Zebrafish Retinal Development: Rescue by Nutritional Supplements. ***Pooja Muralidharan**, James Marrs.* Department of Biology, Indiana University-Purdue University, Indianapolis, Indianapolis, IN.
Fetal Alcohol Spectrum Disorder (FASD) is caused by prenatal alcohol exposure, producing a wide range of defects including neural, ocular and cardiac developmental defects. In particular, severe ocular defects are seen in children diagnosed with FASD including microphthalmia, optic nerve hypoplasia, scotopic vision loss, and coloboma. The zebrafish FASD model recapitulates many defects seen in human patients. Ethanol exposure during early development (cleavage stage through somitogenesis, 2-24 hours post fertilization, hpf) produced severe ocular defects including microphthalmia, optic nerve hypoplasia and photoreceptor differentiation defect. Immunostaining of specific retinal cell types showed severe differentiation defects in photoreceptors, Müller glia, and ganglion cells after ethanol treatment. In situ hybridization showed downregulation of critical transcription factors regulating retinal development such as crx, otx5, and neuroD after ethanol exposure. Increased cell death accounts for the small eye phenotype, and the retina responds with increased proliferation in specific retinal laminae. Dissection of the ethanol-sensitive period identified a developmental stage between 16-24 hpf, which caused severe retinal defects. Interestingly, retinoic acid (RA) or folic acid (FA) nutrient co-supplementation with ethanol during 2-24 hpf or 16-24 hpf rescued photoreceptor differentiation and optic nerve defects. RA rescue of ethanol-induced retinal defects suggest an effect on RA levels in the developing retina, possibly competitive inhibition of RA synthesis by ethanol. RA inhibitor treatment produced retinal defects similar to ethanol-treated retinas. Interestingly, RA supplementation (24-48 hpf or 48-72 hpf) after ethanol treatment (2-24 hpf) restored photoreceptor differentiation, suggesting RA is a missing critical factor for precursor cell differentiation. In contrast, post-treatment with FA, after ethanol treatment, did not restore retinal cell differentiation. FA may function in one-carbon metabolism influencing histone- and DNA-methyl transferase activities, but molecular mechanisms underlying FA rescue of ethanol-induced defects remain unclear.

424. Spatio-temporally controlled signaling modulation in zebrafish. ***A. Felker**, I. Meyer, C. Mosimann.* Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
The heart, blood vessels, and blood arise together with the kidneys and limbs from a common embryonic structure, the lateral mesoderm (LM). The cells of the cardiovascular and hematopoietic system develop within 24 hours post fertilization driven by an as-of-yet unknown combination of extracellular signals and tissue-specific transcription programs. The possible fate-instructing roles of developmental signaling pathways including FGF, RA, Wnt, and BMP in vertebrate organ formation such as the heart have been extrapolated from global embryo perturbations. The emerging challenge is to separate global patterning clues from autonomous fate-instructing mechanisms by achieving tissue-specific genetic perturbations. We use advanced transgenic zebrafish to systematically probe the cell-autonomous roles of developmental signaling pathways in heart, vessel, and blood cell fates. Our strategy combines cardiovascular and hematopoietic creERT2 drivers with transgenes under heat-shock promoter (hsp) and loxP control that express dominant-negative and -active signaling modifiers. The hsp promoter provides temporal control of transgene expression and allows rapid induction of the signaling modifier. The Cre/lox combination provides an additional level of spatio-temporal control via Tamoxifen induction, plus circumvents the general leakiness of hsp-controlled transgenes. Our modular approach allows the rapid cloning of signaling modifiers, transcription factors, and disease-implicated genes under flexible Cre/lox control and spatio-temporal control of their expression based on the selected creERT2 driver line. I established and validated this approach with a loxP transgene driving a dominant-negative version of the FGF receptor (FGFR1-dn). Global induction of our FGFR1-dn transgene using ubi:creERT2 or cre mRNA injection mimics reported phenotypes for FGF inhibition during development. We are now combining our FGFR1-dn transgenics with our growing collection of early LM-specific and cardiac creERT2 driver transgenics to investigate the spatio-temporal requirement of FGF signaling on the stepwise commitment of multi-lineage-potent LM towards cardiovascular cell fates.

425. Development of a Novel Neural Crest EMT Reporter Line for *In Vivo* Screening of EMT and Cell Migration Inhibitors. ***Laura Jimenez***[1], *Jindong Wang*[1], *Robert Solovyev*[1], *Cristhian Toruno*[1], *Tyler Laurent*[1], *Teri Edwards*[1], *David Bearss*[2], *Cicely Jette*[1], *Rodney Stewart*[1]. 1) Department of Oncological Sciences, Huntsman Cancer Institute/University of Utah, Salt Lake City, UT, 84112; 2) Physiology and Developmental Biology, Bringham Young University, Provo, UT 84602.
The Epithelial to Mesenchymal Transition (EMT) plays a crucial role during development and aberrant reactivation of EMT in tumor cells promotes metastasis. Mechanisms that control EMT remain incompletely understood, hampering efforts to target EMT in cancer patients. To identify upstream inducers and downstream effectors of EMT we have developed a novel zebrafish neural crest (NC) EMT reporter line, *Tg(snail1b:GFP)*, for *in vivo* screening of EMT and cell migration inhibitors. After screening a number of candidate kinase inhibitors for their ability to block NC migration in live *Tg(snail1b:GFP)* embryos, we found that the AXL receptor tyrosine kinase (RTK) inhibitor, HCI-2084, potently blocks hindbrain morphogenesis and cranial NC cell migration. Treatment of embryos at the 3-somite stage with HCI-2084 significantly decreases *twist1a* expression, a key regulator of EMT, blocks differentiation of *dlx2a*-expressing chondrogenic precursors, and instead promotes the differentiation of *mitfa*-expressing melanophore precursors that form adjacent to the neural tube. RNA-Seq analysis revealed that HCI-2084 treated embryos have a significant upregulation of retinoic acid (RA) signaling. Co-treatment of HCI-2084 with DEAB, an RA inhibitor, rescues the hindbrain and NC migration and differentiation defects caused by HCI-2084 treatment. We are currently investigating the potential role of AXL in NC cell migration and differentiation, and evaluating HCI-2084 as a possible therapy for neuroblastoma, by using the zebrafish neuroblastoma pre-clinical model and human neuroblastoma cells *in vitro*.

426. Aberrant Autolysosomal Regulation Is Linked to The Induction of Embryonic Senescence in Vertebrate Spns1 Deficiency. *Tomoyuki Sasaki*[1], *Shanshan Lian*[1], *Jie Qi*[1], *Peter Bayliss*[2], *Daniel Klionsky*[3], ***Shuji Kishi***[1]. 1) The Scripps Research Institute, Jupiter, FL; 2) Ontario Cancer Institute, Toronto, ON; 3) University of Michigan, Ann Arbor, MI.
Spinster (Spin) in *Drosophila* or Spinster homolog 1 (Spns1) in vertebrates is a putative lysosomal H^+-carbohydrate transporter, which functions at a late stage of autophagy. The Spin/Spns1 defect induces aberrant autolysosome formation that leads to embryonic senescence and accelerated aging symptoms, but little is known about the mechanisms leading to the pathogenesis *in vivo*. Beclin 1 and p53 are two pivotal tumor suppressors that are critically involved in the autophagic process and its regulation. Using zebrafish as a genetic model, we show that Beclin 1 suppression ameliorates Spns1 loss-mediated senescence as well as autophagic impairment, whereas unexpectedly p53 deficit exacerbates both of these characteristics. We demonstrate that 'basal p53' activity plays a certain protective role(s) against the Spns1 defect-induced senescence via suppressing autophagy, lysosomal biogenesis, and subsequent autolysosomal formation and maturation, and that p53 loss can counteract the effect of Beclin 1 suppression to rescue the Spns1 defect. By contrast, in response to DNA damage, 'activated p53' showed an apparent enhancement of the Spns1-deficient phenotype, by inducing both autophagy and apoptosis. Moreover, we found that a chemical and genetic blockage of lysosomal acidification and biogenesis mediated by the vacuolar-type H^+-ATPase, as well as of subsequent autophagosome-lysosome fusion, prevents the appearance of the hallmarks caused by the Spns1 deficiency, irrespective of the p53 state. Thus, these results provide evidence that Spns1 operates during autophagy and senescence differentially with Beclin 1 and p53.

427. Characterization of actin rich microridges in zebrafish epithelial cells in vivo. ***Pui-ying Lam***[1], *Steve Mangos*[2], *Jochen Reiser*[2], *Anna Huttenlocher*[3]. 1) Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Department of Internal Medicine, Rush University Medical Center, Chicago, IL; 3) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI.
Microridges are present on the apical surface of the outermost epithelial cells in many mucosal surfaces across animal species ranging from human to fish. The function of microridges is not clear but it has been suggested that they provide mechanical strength, retain mucus, and serve as an actin reserve. Microridges are presented in an intricate labyrinth pattern. It is well established that microridges are filled with F-actin. By expressing F-actin bioprobes specifically in epithelial cells in vivo, we have observed that the microridge pattern is dynamic and can change in the order of minutes through breaking and connecting of microridge branches, in addition to de novo synthesis during cytokinesis. However, the molecular and signaling regulation of microridge formation and their actin dynamics is not entirely clear. Here, we characterized the formation of microridges during different developmental stages in zebrafish larvae. Scanning electrometric microscopy showed that microridges appeared as early as 12 hpf. While microridges were present on all surfaces of the zebrafish larvae that were examined, the microridges dissipated in the cornea beginning at 4-5 dpf, only to re-appear at adulthood. In addition, laser wounding and live imaging experiments suggested that changes in cell morphology could induce changes in microridge pattern. Results from actin photoconversion live imaging experiments and inhibitor treatments suggested that the F-actin in microridges is actively treadmilling. Proper actin polymerization, Arp2/3 function, PI3K and Rho kinase activity are all involved in maintaining microridge length. Immunofluorescent staining showed that tyrosine phosphorylated protein is present in microridges. Other actin binding protein such as cortactin and the tight junction protein ZO-1 localized to microridges and suggests that these highly organized and dynamic actin bundles might be related to and regulated in a similar manner to focal and junctional complexes.

428. Generation of a MLIV disease model in zebrafish by genetic modification. ***Huiqing Li***, *Silvia Vergarajauregui*, *Rosa Puertollano*. National Institutes of Health, Bethesda, MD.
Mucolipins are members of a family of endo/lysosomal cation channels that regulate trafficking along the endocytic pathway and whose cellular function is linked to human disease. In mammals, the mucolipin family includes three members, mucolipin-1, -2, and -3 (MCOLN1-3). Loss-of-function mutations in MCOLN1 are associated with a human disease known as mucolipidosis type IV (MLIV). MLIV is an autosomal recessive disease characterized mental retardation, poor muscle function, impaired vision and possible blindness. To better understand the pathology of this disease, we aimed to generate a MLIV disease model in zebrafish. Two putative zebrafish MCOLN1 co-orthologs have been identified, *mcoln1.1* and *mcoln1.2*. By using specific Zinc Finger Nucleases (ZFN), we successfully created two

independent *mcoln1.1* knockout lines. Initial characterization of *mcoln1.1* homozygous null embryos revealed noticeable cell death in the eye. Cell death was confirmed as cell apoptosis by TUNEL staining in both *mcoln1.1* knockout lines. When *mcoln1.1*$^{-/-}$ fish embryos were injected with *mcoln1.2* morpholino, the observed phenotype become even more apparent and increased apoptosis was detected in the whole body of the *mcoln1* lost embryos, thus suggesting some level of redundancy between *mcoln1.1* and *mcoln1.2*. To further confirm these observations, we are currently using the CRISPR/Cas9 system to generate of *mcoln1.2* animals. Overall, our results indicate a novel and unexpected role of mcoln1 during early embryonic development.

429. Interactions between BMP signaling and wdr68 function in craniofacial development. *Greg Alvarado, Robin Shang, Annie Pham, Anish Bhandari, Andrew Martinez, Jessica Wang,* ***Robert M. Nissen***. Biological Sciences, Cal State Los Angeles, Los Angeles, CA.
Many craniofacial syndromes are caused by defects in signal transduction pathways important for D/V patterning of the cranial neural crest cells (CNCCs) that give rise to the upper and lower jaws. For example, defects in Bone Morphogenetic Protein (BMP) signaling are associated with cleft lip/palate, auriculocondylar syndrome is caused by impaired Endothelin-1 (Edn1) signaling, and Alagille syndrome is caused by defects in Jagged-Notch signaling. Accumulating evidence has also revealed complex interactions between the BMP, Edn1, and Jag1b signaling pathways. Specifically, BMP signaling is required for ventral *edn1* expression that, in turn, restricts *jag1b* to dorsal CNCC territory. However, the mechanisms connecting these events are still poorly understood. Previously, we identified the *dcaf7/wdr68* gene as also essential for *edn1* expression. Here, we present the findings that *wdr68* activity is required for jaw development between the 17- to 20-somites stages using a combination of whole-mount IHC and an inducible Tg(hsp70l:GFP-Wdr68) zebrafish line. This window overlaps with the known onset of *edn1* expression at the 18-somites stage. By whole-mount ISH, we found that expression of BMP ligands appear unchanged in *wdr68* mutants. BMP signaling is mediated by the Smad1/5/8 transcription factors and a partner co-Smad4. Interestingly, previous reports detected Wdr68 in physical complexes with multiple Smads suggesting a potential role for *wdr68* within BMP signaling. Consistent with this notion, we further report synergistic reductions of the craniofacial cartilages in *wdr68* mutant embryos that were co-treated with a small-molecule inhibitor of BMP signaling. Conversely, we found that treatment of *wdr68* mutant embryos with a small-molecule agonist for BMP signaling rescued the craniofacial defects. We also found reduced *dlx1a/dlx2a* expression and expansion of *jag1b* and *hey1* expression into ventral CNCC territory of the 1st arch. Thus, *wdr68* appears to mediate *edn1* expression for craniofacial development through facilitating some aspect of BMP signaling.

430. The Role of NR5A2 in Pancreas Development and Carcinogenesis. ***Sahar Nissim***[1]*, Julia Wucherpfennig*[1]*, Xiao-Xu Wang*[2]*, Alec Kimmelman*[2]*, Wolfram Goessling*[1]. 1) Harvard Medical School, Boston, MA; 2) Dana-Farber Cancer Institute, Boston, MA.
Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers today. A recent genome-wide association study (GWAS) has implicated the nuclear receptor NR5A2 in modulating risk for pancreatic cancer. While a role for this gene in pancreatic cancer has not been previously recognized, it is known to have an important role in stem cell pluripotency and metabolism. Using the zebrafish model organism, we demonstrate that knockdown of nr5a2 results in absence of exocrine pancreas, while leaving endocrine pancreas unaffected, indicating an essential role in pancreas organ formation. Further, we localize expression of nr5a2 to the endodermal bud that gives rise to the exocrine pancreas, consistent with a role for nr5a2 in regulating differentiation of pancreas progenitors. In resected human PDAC specimens and in vitro cell lines, we find that NR5A2 expression is altered compared to normal ductal epithelium. Moreover, shRNA knockdown of NR5A2 dramatically alters proliferation of PDAC cell lines, supporting the hypothesis that NR5A2 is involved in pancreatic carcinogenesis. These data support a model that NR5A2 regulates the proliferation and differentiation of exocrine pancreas progenitor cells during development. Consistent with this model, we find that signaling through nr5a2 interacts with notch activity. We postulate that aberrant expression of NR5a2 results in dysregulation of these processes in the adult pancreas by driving ductal epithelial cells to acquire properties of progenitor cells, and therefore is an important step in pancreas oncogenesis.

431. Is it a vibrissa? Anatomy and ontogeny of a novel hemodynamic organ in zebrafish. *Erica A. Binelli, Alejandra N. Luna,* ***Elizabeth E. LeClair***. DePaul University, Chicago, IL.
The zebrafish maxillary barbel (ZMB) can protract and retract in response to stimuli; however, the mechanism of barbel movement is not known. Using whole-mount phalloidin staining of the proximal ZMB, we observed an erythrocyte-filled sinus surrounded by 'barrel-staves' of filamentous actin, suggesting highly organized vascular smooth muscle cells (VSMCs). Although the sinus is variably filled by erythrocytes in vivo, cardiac injection of fluorescent dextrans shows that this chamber consistently contains plasma. Full-thickness confocal imaging of dextran-injected adults containing EGFP^{+} endothelial cells *(Tg(fli1a:EGFP))* revealed a vascular complex with three compartments, here named the distal bulb, central chamber, and accessory chamber. The early ontogeny of all three compartments was examined in a whole-mount series of *(Tg(fli1a:EGFP))* juveniles; in wild type adults, each compartment was studied using histology, immunohistochemistry and transmission electron microscopy (TEM). The sinus develops from a loose vascular plexus within the dermis lateral to the maxillary bone. This plexus remodels to form three chambers, each of which has a unique arrangement of extracellular matrix and perivascular cells. The distal bulb fits within a concavity in the ZMB's central rod, forming a close connection to the barbel proper. The large central chamber has thick muscular walls with luminally-elongated septa, forming semi-detached blood-filled lacunae. Both the walls and the septa are densely innervated by small, unmyelinated axons. The accessory chamber appears neither innervated nor muscularized, but is an endothelial cul-de-sac with a thick elastic adventitia, suggesting an extensible fluid reservoir. We propose that we have identified a new organ in zebrafish, here named the maxillary barbel blood sinus, which is responsible in part for the movement of this appendage. Additionally, we speculate that the mechanical connection between the barbel base, the blood sinus, and the densely innervated VSMCs may represent a previously undescribed hemodynamic sensory system, analogous to mammalian facial vibrissae.

432. Profiling the in vivo endothelial translatome using RIboTag zebrafish. ***Mayumi Miller***, *Kathryn Monzo*, *Brant Weinstein*. Program in Genomics of Differentiation, NICHD, Bethesda, MD.
Recent advances in transcriptomics have allowed for expression profiling of actively transcribed genes by sequencing the RNA (RNA-seq) expressed in collections of cell and tissue samples. By comparing cell and tissue samples from wild type and mutant or experimentally manipulated backgrounds, the signaling pathways and proteins involved in multiple developmental contexts can be interrogated. However, obtaining a homogenous sample for analysis has proven challenging, and the methods to isolate specific cell types, such as the endothelial cells of the vasculature, introduce artifacts and confounding factors to transcriptome analysis. Collection of endothelial cells requires methods such as laser capture dissection, FACs sorting, or gross dissection, and these methods are time consuming and/or activate stress response programs. These extrinsic manipulations may obscure the native transcriptional state, and therefore interfere with downstream analysis of the transcriptome. Recent work in the mouse utilizing tagged ribosomal proteins illustrated a novel approach to collect actively translated transcripts, with minimal manipulation to the cells prior to collection of the RNA. Implementing this technology in the zebrafish, we have generated a "RiboTag" transgenic line in which an endothelial cell specific promoter drives expression of a tagged ribosomal subunit. This transgenic line permits purification of actively translated mRNAs specifically in endothelial cells. By collecting whole or grossly dissected zebrafish embryo lysates, and performing immunoprecipitation for the tagged ribosomal subunit, we can specifically collect RNAs being actively translated in endothelial cells. Our initial work confirms robust enrichment of endothelial cell markers in immunoprecipitated RNA compared to total RNA. The ribotag line provides a highly valuable tool to rapidly, easily, and consistently query endothelial gene expression at different stages of development and in different mutants or functionally manipulated animals. This new tool should help to greatly enhance our understanding of the molecular regulation of endothelial development.

433. Screening of functional "endo-miRNAs" using zebrafish genome targeting mutagenesis strategies. ***Anand Narayanan***[1], *Guillermina Hill-Teran*[1], *Emma Ristori*[1], *Yong Kong*[2], *Stefania Nicoli*[1]. 1) Yale Cardiovascular Research Center, Yale University, New Haven, CT; 2) Keck Biotechnology Center, Yale University, TAC building, 06511, CT, USA.
Formation of blood vessels are required for organ growth and homeostasis during embryonic development as well as in disease-related processes like tumor growth, wound healing, and restoring blood flow to tissue after injury. Since the signaling mechanisms required for blood vessel formation are conserved and are similar during embryonic and adult stages, it is possible to utilize model organisms to investigate this process. In particular, the zebrafish has proven to be a powerful model for the study of vascular development. The genetic accessibility and transparency of Zebrafish embryos has allowed novel discoveries into endothelial cell behavior through detailed visualization of blood vessel growth as it occurs in vivo. MicroRNAs (miRNA) are small non-coding RNAs that can negatively regulate gene expression by blocking mRNA translation and/or stability. Recent evidence demonstrates their importance for proper growth and differentiation of many cell types, including endothelial cells. Indeed, a few miRNAs termed endo-miRNAs such as miR-126 and miR-221 showed high endothelial expression in vivo and play major roles in vascular development and homeostasis. Here, we identified via Illumina deep sequencing the entire repertoire of zebrafish endo-miRNAs expressed in embryonic and adult vascular system. This screening showed that a limited number of miRNAs are specifically expressed in endothelial cells during development and adult life. Several others miRNAs are found enriched but not exclusively expressed within the vascular tissue questioning their specific cardiovascular role. In order to assay zebrafish endo-miRNAs function we are applying Tal-Effector Nucleases (TALENs) and CRISPR-CAS9 gene mutagenesis strategies in order to target functional regions of single and/or multiple endo-miRNAs mature sequences. In this poster we summarize an update of our work.

434. Reverse genetic screening via CRISPR-CAS9 mutagenesis of endo-miRNAs: a novel strategy to identify tissue specific miRNA function. *A. Narayanan, E. Ristori, Y. Kong, **S. Nicoli***. Yale Cardiovascular Research Center, New Haven, CT.
microRNAs (miRNAs) have a pivotal role during gene regulation at the post-transcriptional level and impact the cardiovascular function during physiological and pathological conditions. The continue advances in RNA technology and bioinformatic approaches have improved the ability to dissect complex signaling pathways driven by miRNAs. More than ~500 miRNAs have currently been found within the mammalian genome, however only a few specific miRNAs, named endo-miRNAs, showed conserved endothelial cell expression and have a recognizable vascular function. Indeed classical genetic ablation of a specific miRNA gene often does not result in severe defects during embryonic development. Many miRNAs exist as duplicates or share identical seed regions with other miRNA families, thus there remains the question of functional redundancy. Here we pioneer the possibility to perform reverse genetic screening in non-coding RNA sequence targeting a large number of tissue specific miRNAs. Using next generation sequencing, we performed a comprehensive miRNA sequence analysis of primary zebrafish and human vascular cells. Thus, we identified ~50 miRNAs with conserved endothelial expression. To screen their function in vivo we created a high throughput CRIPSR-CAS-9 strategy using multiple guide RNAs (gRNAs) to target the mature miRNA sequences of endo-miRNAs genes. Multiple vascular phenotypes resulted from the injection of our gRNA-endomiRNAs library, including intracranial hemorrhages, angiogenesis and vascular remodeling defects. Further analysis will be necessary to identify and validate the genomic mutations within the specific miRNA. In summary, our strategy formally proves the possibility to perform reverse genetic screening for tissue specific miRNAs function.

435. Transcriptional control of etv2 expression during embryonic cardiac development. *Marcus-Oliver Schupp*[1], *Maathew Waas*[1], *Chang Zoon Chun*[2], ***Ramani Ramchandran***[1]. 1) Pediatrics, Medical College of WI, Milwaukee, WI; 2) University of Florida.
During vertebrate embryonic development, cells of the cardiac, blood and vascular lineages are specified from lateral plate mesoderm (LPM) via integration of inductive signals and systematic activation and deactivation of transcriptional programs in progenitor cells. E26 transformation-specific domain (Ets) variant 2 (Etv2) factor function is necessary for repression of cardiac fate in putative endothelial-fated precursor cells (Palencia-Desai et al., 2011) suggesting the existence of a common progenitor cell of cardiac, blood and vascular lineages. We hypothesized that examination of etv2 gene regulation will reveal mechanisms involved in the differentiation of sub-populations of

blood, endothelial and cardiac cells from anterior LPM in zebrafish. Two studies (Proulx et al., 2010; Veldman and Lin, 2012) have already identified genomic fragments that control tissue-specific etv2 expression during zebrafish embryonic development. Proulx et al. reported elements in the etv2 genomic locus responsible for vascular-specific expression. Veldman et al. identified a 110 bp upstream enhancer of etv2 that contains a functional binding site for Foxc1a and Foxc1b factors responsible for vascular-specific etv2 expression. Here, by using transgenic reporter analysis of the etv2 genomic locus, we identified cis-regulatory elements that are responsible for the transcriptional repression of the etv2 gene in precursors of endocardial and myocardial cells. Using morpholino oligonucleotide-mediated knockdowns, we found that Scl and Nkx2.5 transcription factors participate in this repression mechanism. Cardiac inhibition of etv2 expression is likely through sequence-specific binding of Scl to the etv2 promoter as shown in a protein-DNA interaction assay. Moreover, using a dual-luciferase reporter system, we identified cis-regulatory elements located in the first two introns of etv2. Together, our studies reveal evidence for developmental plasticity of a progenitor cell of endothelial, endocardial and myocardial lineages that responds to etv2 expression modulation during embryonic cardiovascular development.

436. Alk1 is required for directed cranial arterial endothelial cell migration. ***Elizabeth R. Rochon***, *Sarah Young, Beth L. Roman.* Biological Sciences, University of Pittsburgh, Pittsburgh, PA.
In the normal vasculature, arteries and veins are connected by capillaries that facilitate the exchange of nutrients and oxygen to surrounding tissues. In rare events, arteries connect directly to veins, forming arteriovenous malformations (AVMs). These fragile, tortuous malformations can lead to hemorrhage, ischemia, and stroke. Haploinsufficiency of one of several TGF-beta signaling pathway components underlies hereditary hemorrhagic telangiectasia (HHT), a family of genetic disorders characterized by a predisposition to AVM development. Heterozygous mutations in ALK1, which encodes an arterial endothelial cell (EC)-specific TGF-beta type I receptor, are implicated in HHT2. However, the mechanism by which decreased ALK1 function leads to AVMs is unknown. To better understand the function of ALK1 in arterial ECs, we compared endothelial cell behavior in control embryos and zebrafish alk1 morphants, which develop cranial AVMs. In wild type embryos, alk1-positive cranial arterial ECs exhibit a dramatic net migration toward the heart between 24-48 hpf. In contrast, the migration of cranial arterial ECs in alk1 morphants is significantly shifted away from the heart, resulting in increased EC number in distal cranial arteries and decreased EC number in proximal cranial arteries. Similar although more dramatic effects on cranial EC migration were noted in tnnt2a morphants, which lack heartbeat and blood flow. These results suggest that EC migration toward the heart is heartbeat- and/or blood flow-dependent, and that loss of Alk1 signaling, which we have previously shown to be a flow-dependent signaling pathway, impairs this response. This impaired response results in a redistribution of arterial ECs away from the heart, leading to enlarged distal arteries and, consequently, downstream AVMs as a result of altered hemodynamics. Current studies are focused on defining the biochemical or mechanical factors that govern flow-dependent arterial EC migration toward the heart.

437. Effects of the loss of DNA polymerase a subunits on nuclear atypia and apoptosis. ***Alex Y. Lin***, *Anna Le, Kristin Eckert, Keith C. Cheng.* Jake Gittlen Cancer Research Foundation and Division of Experimental Pathology, Penn State Hershey College of Medicine, Hershey, PA 17033.
Histologically-scored cellular atypia is associated with human cancers and precancers. This cytological feature is used in the diagnosis of multiple human cancers. Due to the diagnostic importance of this phenotype, we have studied a mutant from a forward genetic screen that exhibits nuclear atypia. The zebrafish mutant *huli hutu* (*hht*) is an ENU-derived larval-lethal mutant that exhibits nuclear atypia and an apoptotic phenotype that is tissue-specific. The causative mutation is an AT-insertion in exon 2 of *pola2*, which encodes subunit B of DNA polymerase a. Similar phenotypes have been observed in mutants in two other subunits of Pol a, *pola1* (a retroviral insertional allele from the Hopkins collection) and *prim1* (a F110S missense allele in a mutant known as *piy*). As part of our investigation of the relationship between DNA polymerases and nuclear atypia, we report the phenotypes of morphants and CRISPR/Cas-9 knockouts of all four subunits of DNA polymerase a to assess the tissue specificity of phenotypes.

438. Dissecting the molecular mechanisms of myelination through a high-throughput forward genetic screen. ***Breanne L. Harty***, *Sarah E. DeGenova, Amy L. Herbert, Charleen L. Johnson, Kelly R. Monk.* Washington University - St Louis School of Medicine, Saint Louis, MO.
Myelin is a multilamellar sheath made by specialized glial cells that insulates and protects axons in the vertebrate nervous system. These myelinating glial cells are Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS). Myelination facilitates rapid action potential propagation and provides trophic support for proper functioning and survival of neurons in vertebrates. Disruption of myelination results in debilitating symptoms in many neurological diseases, including multiple sclerosis in the CNS. Unfortunately, we lack effective therapies to prevent demyelination and/or promote remyelination, and our poor understanding of how myelin is formed, maintained, and utilized for axonal survival hinders development of new therapies. Understanding the molecular mechanisms that govern myelination thus has direct consequences for human health. Forward genetic screens in zebrafish have successfully identified important conserved regulators of myelination such as *gpr126* and *kif1b*, although previous screens were not performed to saturation. To define new regulators of myelination, we have performed an F3 forward genetic screen using transgenic zebrafish expressing fluorescent reporters driven by the *lhx1a* and *mbp* promoters, which mark neurons and myelinating glia, respectively. To date, we have screened over 600 genomes and recovered 15 heritable myelination mutants, as well as at least 10 additional putative mutants. Two heritable mutants, *stl64* and *stl90*, have very striking and distinct phenotypes in the CNS. *stl64* mutants show dramatic overexpression of *mbp*, while *stl90* mutants almost completely lack *mbp* expression. Our ongoing work, including mutant gene identification using whole genome sequencing, marker analyses, transmission electron microscopy, and in vivo time-lapse imaging will be discussed.

439. Developing a transit assay of gut motility in Zebrafish. ***Caitlin Farris***, *Julie Kuhlman*. Iowa State University, Ames, IA.
Hirschsprung's disease is a disorder in which the enteric neurons fail to migrate to the lower end of the gastrointestinal tract during development. This disease has been linked to mutations in SRY-box containing gene 10 (Sox10). A zebrafish lacking Sox10 function (cls) has been generated to further understand Hirschsprung's disease. This fish lacks enteric neurons, but still has the ability to pass food. To examine the gut motility of these organisms, this lab is developing a transit assay using fluorescent beads. The addition of the chemical dextran sulphate sodium (DSS) appears to increase transit time and could be instrumental in developing a method to measure differences in transit rate between zebrafish models.

440. Identification of markers for enteric neurons and interstitial cells of Cajal (ICC) in the Zebrafish Gastrointestinal tract. ***Kevin Natukunda***[1], *Pal Narinder*[2], *Sweta Roy*[1], *Caitlin Farris*[1], *Julie Kuhlman*[1]. 1) Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA; 2) Department of Agronomy, Iowa State University, Ames, IA 50011, USA.
Zebrafish has emerged as a better model organism to study vertebrate development, as most organ systems, physiology and molecular pathways are well- conserved between mammals and zebrafish. By 5 days post fertilization (dpf), most major organ systems are present in Zebrafish larvae. Apart from being used to study vertebrate development, the zebrafish is also used to understand human disease conditions such as functional gastrointestinal (GI) disorders. Multiple cell-types integrate to ensure proper performance, regulation and functioning of the Gastrointestinal (GI) tract, including: Interstitial cells of Cajal (ICC), enteric neurons, and smooth muscle cells. This complex process involves interaction, communication and cooperation of these many cell types. The goal for this project is to identify cell specific markers for these different cell types and to gain a more complete understanding of the spatial and temporal expression patterns of these cells through enteric nervous system development.

441. Spontaneous excision of *ZesT*, a novel, endogenous transposable element of zebrafish in the Enhancer/Suppressor Mutator (En/Spm) superfamily. ***ZR Tsetskhladze***[1], *JF McCormick*[2], *VA Canfield*[3], *S Johnson*[4], *DJ Grunwald*[2], *C Feschotte*[2], *KC Cheng*[1]. 1) Jake Gittlen Cancer Research Foundation and Division of Experimental Pathology, Penn State Hershey College of Medicine, Hershey, PA; 2) Department of Human Genetics, University of Utah, Salt Lake City, UT; 3) 31525 Bradley Ave., Hummelstown, Pa; 4) Department of Genetics, Washington University, St. Louis, MO.
We have found evidence of excision of a novel transposable element in zebrafish. Sequencing of *slc45a2*, the zebrafish orthologue of the human *OCA4* gene, in *albino*^{b4} zebrafish revealed a 4 kb insertion in exon 6 (Tsetskhladze, et al., 2012, *PLoS One* 7: e47398). Organization of the insertion sequence resembles that of the Enhancer/Suppressor Mutator (En/Spm) superfamily of DNA elements originally described in maize. Genotyping of *albino* progeny originating from *albino*^{b4} fish from the Johnson laboratory revealed two excision alleles centered at the original point of the insertion allele. Excision resulted in 2 or 6 nucleotide deletions in the coding sequence, indicating imprecise excision of the inserted element. Since no complete transposase is present in the original insertion, we expect excision to have been mediated in trans from elsewhere in the genome. We found 12 representations of the insertion sequence in zebrafish reference genome - *ZV9*. As far as we are aware, this is the first evidence of active excision of an endogenous transposable genetic element in zebrafish. We are calling these elements Zebrafish En/Spm Transposon elements (*ZesT*) due to their high sequence similarity with En/Spm. We will review evidence of excision events and the structure of *ZesT* elements found in the genome.

442. Temperature-sensitive splicing of the *mitfa vc7* allele. *Zhiqiang Zeng*[1], *E. Elizabeth Patton*[1], ***James Lister***[2]. 1) MRC Institute of Genetics and Molecular Medicine, MRC Human Genetics Unit & Edinburgh Cancer Research Centre, Edinburgh, UK; 2) Department of Human and Molecular Genetics and Massey Cancer Center, Virginia Commonwealth University, Richmond, VA USA.
The *mitfa* gene encodes a zebrafish ortholog of the microphthalmia-associated transcription factor (Mitf) which, like its counterparts in other species, is absolutely required for development of neural crest melanocytes. We previously identified a hypomorphic allele of *mitfa* which is temperature-sensitive for melanocyte development. The *mitfa vc7 ts* allele is a mutation in a splice donor site that reduces the level of correctly-spliced transcripts. Temperature upshift experiments with *mitfa vc7 ts* show that *mitfa* is required at several stages of melanocyte differentiation, including for expression of the early melanoblast marker *dct*, again for progression from *dct* expression to differentiation, and again for maintenance of dendritic form following differentiation (Johnson et al. 2011, *Dev. Biol.* 350:405-13). We have also found that *mitfa vc7 ts* cooperates with a melanocyte-expressed *BRAF(V600E)* transgene to promote melanoma that reflects the pathology of the human disease (Lister et al. 2014, *J. Invest. Dermatol.* 134:133-40). We have investigated the basis of the temperature-sensitivity of the *vc7* allele and find that its splicing is temperature-dependent. Correct splicing occurs at low (permissive) temperature while mis-spliced products predominate with increasing temperature. The mis-spliced mRNAs include those that skip exons or retain introns while preserving the reading frame; the isoforms thus encoded are unable to rescue melanocytes in *mitfa* null mutant embryos and at least one may have antimorphic activity. We are presently investigating if the temperature-dependent splicing mechanism of the *vc7* allele can be exploited for conditional regulation of heterologous gene expression.

443. Development of Novel Cell-based and Zebrafish Transgenic Strategies to screen for KIT dimerization inhibitors. ***Vinothkumar Rajan***[1,2], *Sergey Prykhozhij*[2], *Jason Berman*[1,2]. 1) Microbiology/Immunology, Dalhousie Univeristy, Halifax, NS, Canada; 2) Department of Pediatrics, IWK Health Center, Halifax, NS, Canada.
KIT is a tyrosine kinase receptor protein expressed in many tissues. KIT is implicated in various cancers including acute myeloid leukemia, gastrointestinal stromal tumor, and melanoma and also in hematological disorders like mastocytosis. KIT becomes functionally active when two KIT monomers join to form a KIT dimer upon ligand binding. The D816V KIT mutation results in KIT dimerization in the absence of ligand and KIT protein phosphorylation. Using protein disorder prediction algorithms and in silico peptide simulation-based analysis of the KIT mutational hotspot, we determined that dimerization of KIT D816V results in increased protein stability. In addition to D816V, other frequent oncogenic mutations at the mutation hotspot were also capable of stabilizing KIT. Given that the D816V mutation makes KIT

intracellular domains dimerize, dimerization inhibitors represent a potential targeted therapeutic approach in diseases with mutant KIT. We developed an in vitro cell line-based screening assay that uses bimolecular fluorescence complementation to screen dimerization inhibitors of KIT using fluorescence emission. This in vitro screening approach will be complemented by a similar in vivo assay in the zebrafish model. We previously developed transgenic zebrafish expressing a human KIT D816V mutation that developed a mastocytosis like phenotype demonstrating conserved function of KIT and the mutation in the zebrafish model. We are developing a transgenic zebrafish using the Tet-ON-tool kit system. Expression of KIT-BiFC will be controlled by a tetracycline-activated transcription factor driven by microphthalmia-associated transcription factor a (mitfa) promoter. We are also developing novel peptide-based dimerization inhibitors, which will be tested in both cell and zebrafish reporter assays. Ultimately, these platforms will reveal novel dimerization inhibiting compounds that may have a significant impact on treating diseases with KIT mutations as well as other tyrosine kinases having similar activation mechanisms.

444. Roles of slc24a5 in UV induced DNA damage response and melanoma onset. ***Kajan Ratnakumar***[1], *Richard White*[1,2]. 1) Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY; 2) Weil Cornell Medical College, New York, NY.
Cutaneous malignant melanoma has a stronger connection to ethnicity than most cancers. People of European ancestry are far more susceptible to the disease than people from any other region and the link between this susceptibility and ethnic heritage has led to the view that alterations in melanin production predispose individuals to cutaneous melanoma onset. A single nucleotide polymorphism in the slc24a5 gene has been implicated in skin color variation in human populations - people of European ancestry carry a variant or derived allele of the gene (T111) while non Europeans carry an ancestral version (A111). The slc24a5 gene encodes a trans-golgi membrane protein, NCKX5, involved in melanosome maturation. Intriguingly, the zebrafish golden mutant was shown to bear a nonsense mutation in the slc24a5 gene leading to its lack of pigmentation (incurred primarily via a loss of melanocyte and melanosome structure and function). In this regard, the golden mutant essentially resembles the skin structure of humans of European descent, who are highly susceptible to melanoma. In order to decipher the role of melanin with regard to UV induced DNA damage and melanoma onset, we have been treating golden zebrafish embryos with UVB and comparing their response to the respective wild type fish. Preliminary data from our lab suggests (1) golden mutants are unable to regulate p53 levels upon UV treatment and further, (2) golden mutants display an overall greater sensitivity to UV in terms of the resultant morphological abnormalities and death. We are hence interested in interrogating the function of slc24a5 with regard to UV sensitivity and DNA damage sensing. We are testing the hypothesis that slc24a5-mediated melanin production is one factor involved in the protection of melanocytes from transformation via UV damage, potentially via activation of the p53 pathway. Furthermore, we are testing the role of slc24a5 in melanoma by crossing into the existing zebrafish melanoma model (mitfa-BRAFV600E;p53-/-) and assessing sensitivity to UV exposure. We anticipate this work will lead to an understanding of the interplay between ethnicity, UV induced DNA damage and melanoma onset.

445. Identification of a zebrafish germline mutation that causes T cell cancer and apoptosis resistance. ***Barbara Squiban***, *J. Kimble Frazer*.
Section of Pediatric Hematology/Oncology, Department of Pediatrics, OUHSC, Oklahoma City, USA.
Leukemia and lymphoma are the 1st and 3rd most common pediatric cancers, and T cell cancers are common subtypes of both entities. Our group used a forward-genetic screen to create mutants prone to T cell cancer. The screen was performed using transgenic fish expressing a T cell-specific GFP reporter, thus, GFP+ thymic tumors detect fish with T cell cancer. One mutant line, oscar the grouch (otg), shows recessive cancer predisposition, and their cancers are resistant to treatments that normally induce T cell apoptosis. Larvae with bi-allelic otg mutation also have defects in radiation-induced apoptosis. My project seeks to identify the mutation in otg fish using this larval phenotype. I will use RNA-seq to find SNPs linked to otg. RNA-seq will reveal otg's candidate genomic locus and also yield information on downstream effectors in the otg pathway. Thus, if otg is a gene that impacts the apoptotic pathway as we hypothesize, identifying otg will inform our understanding of apoptosis and how this germline mutation contributes to T cell oncogenesis.

446. Error-Prone DNA Polymerases in the Genetic Evolution of Melanoma. ***Kelsey Temprine***[1], *Erin Langdon*[2], *Richard M White*[1]. 1) Department of Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Dartmouth College, Hanover, NH.
Background: Melanoma is the 5th most common type of cancer, and MAPK pathway activation is central to the disease, making it a key druggable target. However, despite the establishment of targeted inhibitors with profound activity in patients, their ultimate effectiveness is limited as resistance quickly develops. Thus, the development of drug resistance is a significant hurdle in the treatment of cancer and long-term survival of patients. Drug resistance may occur due to pre-existing genetic heterogeneity or due to the acquisition of new mutations after the pressure of drug is applied. In bacteria, the acquisition of new mutations under selection is referred to as stress-induced mutagenesis (SIM), which is mediated by upregulation of error-prone DNA polymerases such as DinB. We hypothesize that a similar mechanism of SIM exists in cancer via upregulation of the error-prone DNA polymerase polk (the vertebrate homologue of DinB).
Methods: Human melanoma cells (A375) were treated with a BRAF inhibitor for 24 hours until 4 months. Levels of polk and related Y-family polymerases were measured using Q-RT-PCR. Concomitantly, DNA damage was measured using staining for gH2AX. **Results:** Both acute (24 hours) and chronic (1 month) treatment of A375 cells led to a greater than 4-fold increase in the expression of polk along with moderate increases in the other Y-family polymerases. However, by 4 months of treatment when cells had become resistant, the levels of polk returned to normal. The levels of DNA damage were not significantly increased by BRAF inhibition. Similar approaches are being taken in other human cell lines and melanoma-prone transgenic zebrafish (mitf-BRAF^{V600E};p53$^{-/-}$). **Conclusions/Future Plans:** The upregulation of error-prone DNA polymerases in melanoma would provide a mechanism linking cellular stress to the acquisition of new mutations, a form of evolvability in cancer. We plan to test the mechanisms by which BRAF inhibition leads to upregulation of polk and determine the functional consequences of polk upregulation in terms of mutational spectrum (RADseq) and drug resistance.

447. Using Zebrafish Xenotransplantation to Evaluate Anti-Cancer Drug Efficacy: Examining the Impact of Focal Adhesion Kinase Inhibition on Ewing's Sarcoma Cell Proliferation and Metastasis. ***Chansey Veintotte***[1], *Brian Crompton*[2], *Nicole Melong*[1], *Katherine Fraser*[1], *Poul. B Sorensen*[3], *Graham Dellaire*[4], *Kimberly Stegmaier*[2], *Jason N. Berman*[1]. 1) Department of Pediatrics, IWK Health Centre, Halifax, NS, Canada; 2) Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts; 3) Department of Pathology, University of British Columbia, Vancouver, BC, Canada; 4) Department of Pathology, Dalhousie University, Halifax, NS, Canada.
Although many therapeutic advances have been made for human cancers over the last decade, treatment options for metastatic Ewing's Sarcoma (ES) remain static. One factor shown to contribute to the expansion and survival of the ES is expression/activity of Focal Adhesion Kinase (FAK). This tyrosine derived kinase functions to increase cell adhesion, cell proliferation and may have a role in cell migration. Xenotransplantation (XT) of human cancer cells into transparent zebrafish embryos provides a versatile tool for evaluating interactions between tumour cells and the micro-environment. Zebrafish XT offers a rapid means to screen compounds for anti-cancer activity and prioritize which agents should be explored further using traditional murine models. Human ES cell lines (TC32 & A673) were fluorescently labeled and injected into yolk sacs of two-day-old casper embryos. Cell proliferation was monitored by live-cell microscopy and quantified in single cell suspensions by computer-based software. Cell migration was examined by observing pre-defined tail regions every 24h to determine the presence of ES cells. ES cells survived, proliferated and migrated to the tail tissue in zebrafish embryos. Upon treatment with FAK inhibitor PF-562271 we observed a reduction in cell proliferation, resembling the results obtained during in vitro experiments. Migration of TC32 ES cells was not inhibited by drug treatment. These results highlight the ability of the zebrafish XT platform to de-risk pre-clinical studies by providing "go/ no-go" decisions regarding therapies. Further validation is underway to examine the impact of FAK inhibition on other ES cell lines, in combination therapy and the efficacy of other FAK inhibitors.

448. Stimulation of hepatocarcinogenesis by neutrophils in oncogenic kras-induced hepatocellular carcinoma model in zebrafish. ***Chuan Yan***[1,2], *Xiaojing Huo*[2], *Zhiyuan Gong*[1,2]. 1) NUS Graduate School for Integrative Sciences and Engineering; 2) DBS, NUS.
In the past few years, increasing evidence has indicated a tumor promoting role of neutrophils in carcinogenesis. Recently our laboratory has developed an inducible, HCC model by transgenic expression of oncogenic $kras^{V12}$ and this transgenic line provides an excellent model for investigating tumor initiation. To study the role of neutrophils in hepatocarcinogenesis, we crossed the $kras^{V12}$ transgenic line with Tg(lysC:DsRed) that has DsRed expression in neutrophils and observed a rapid migration of neutrophils to the $kras^{V12}$ expressing hepatocytes. By pharmaceutical stimulation of neutrophil activity, we found increases of both number and density of neutrophils in oncogenic livers, accompanied with a further increase of the liver size. Conversely, pharmaceutical inhibition of neutrophil activity caused decreases of neutrophil number and density in oncogenic livers as well as a corresponding decrease of oncogenic liver size; similar observation was also confirmed by inhibition of neutrophil differentiation through morpholino knockdown of Granulocyte colony-stimulating factor receptor. The increase of oncogenic liver size by neutrophils was also accompanied by increase of cell proliferation and decrease of apoptosis, and vice verse. These tumor infiltrated neutrophils (TINs) displayed a stagnant migratory pattern in the liver tumor, meandering along the tumor edges, as compared to naïve neutrophils. Furthermore, TINs were isolated via fluorescence-activated cell sorting (FACS), showing several distinct features, including 1) enriched hyper-segmented nuclei; 2) decreased reactive oxygen species and myeloidperoxidase activity. Molecular analyses of FACS isolated hepatocytes and TINs indicated changes of several important molecular pathways, including promotion of a pro-inflammatory microenviroment in oncogenic hepatocytes by increased tgf-b1a expression and down-regulation of tnf-a and ifng, whereas in TINs, there was a decrease of expression of anti-tumor cytokines such as tgf-b1b and il-10. Collectively, our data suggest a promoting role of neutrophils in early hepatocarcinogenesis in zebrafish after the initial response to inflammatory cue from oncogenic livers.

449. Screening for Neural Crest Lineage Factors Hijacked during Melanoma Metastasis. ***Yan Zhang***[1,2], *Kajan Ratnakumar*[1], *Silja Heilmann*[1], *Erin Langdon*[1], *Emily Kansler*[1], *Charles Kaufman*[3], *Christian Mosimann*[4], *Leonard Zon*[3], *Joao Xavier*[1,2], *Richard White*[1,2]. 1) Memorial Sloan Kettering Cancer Center, New York, NY; 2) Weill Cornell Medical College, New York, NY; 3) Dana-Farber Cancer Institute, Boston Children's Hospital, Boston, MA; 4) Institute of Molecular Life Sciences, University of Zürich Winterthurerstrasse, Zürich , Switzerland.
Background: Melanoma is an aggressive disease, with metastasis accounting for nearly all deaths from the disease. Tumor metastasis can be viewed as a multistage process whereby malignant cells disseminate itself from origin and colonize a distal organ. The capacity for metastasis may be acquired (due to novel genetic/epigenetic changes) or intrinsic (due to migratory programs embedded in the differentiation history of the lineage). Because melanoma is derived from the migratory neural crest, we wish to identify lineage-specific genes that contribute to the intrinsic metastatic capacity of melanoma. ***Methods***: We are testing neural crest lineage factors as mediators of metastasis in the zebrafish melanoma model. These genes are being tested in two complementary assays: 1) transplantation of the zebrafish cell line ZMEL1 (overexpressing genes of interest) into the casper recipient, and 2) creating mosaic transgenic animals with the gene of interest expressed on the background of the mitfa-BRAFV600E;p53-/- melanoma-prone line. ***Results***: Methods to nucleofect genes in the ZMEL1 have been optimized, allowing for expression of any gene of interest. Quantitative assays of metastatic capacity have been developed using image analysis. Candidate genes (SNAI2, WNT5A, NEDD9) are being tested using the casper assay. For creation of the transgenic fish, we have created an expression vector in which the candidate gene is driven by the mitfa promoter, along with a crestin-tdTomato marker which is relatively tumor specific in the BRAF;p53 line. This will allow for visualization of both primary tumor and metastasizing cells. ***Future plans***: Optimization of both assays will allow for rapid identification of neural crest lineage factors as mediators of melanoma metastasis in the zebrafish, which will be complemented by data from human melanoma samples.

450. Estrogens regulate embryonic heart rate via a G-protein coupled receptor. ***Shannon N Romano***, *Muhan Hu, Daniel A Gorelick.* Pharmacology and Toxicology , University of Alabama at Birmingham , Birmingham , AL.
Sex differences in resting heart rate in humans correlate with sex differences in circulating levels of estrogens. Whether estrogens regulate heart rate directly, and by what mechanism, is not well understood. Estrogens regulate gene expression by binding to the nuclear hormone receptors ERa and ERb, which are ligand-dependent transcription factors. Estrogens can also modulate cell function rapidly, independent of gene expression, via membrane-associated G protein-coupled estrogen receptors (GPERs). It is assumed that estrogen signaling in the heart is mediated by nuclear receptors, but this hypothesis has not been tested directly. We used a pharmacological approach to study the effects of estrogens on heart rate in zebrafish embryos. At 50 hours post fertilization, embryos exposed to water containing formestane, an aromatase inhibitor that reduces estradiol synthesis, displayed reduced heart rate compared to controls. This effect was rescued by estradiol, an ERa/ERb/GPER agonist, and by ICI182,780 (ICI), a well-characterized ERa/ERb antagonist and GPER agonist. Rescue of heart rate by ICI strongly implicates GPER and excludes ERa and ERb signaling. Additionally, the effects of formestane were reversed by co-administration of a membrane impermeable estrogen (estradiol-BSA conjugate), suggesting that estrogens act at the plasma membrane to regulate heart rate. Exposure to estradiol, estradiol-BSA or ICI alone increased heart rate within 30 minutes, consistent with rapid estrogen receptor signaling at the plasma membrane. To identify potential cells in which GPER acts, we performed in situ hybridization on 1-5 day old zebrafish. *gper* transcripts were detected in discrete regions of the brain but were not detected in the heart, suggesting that estrogens regulate heart rate via GPER signaling in the brain. To test this hypothesis, we are using CRISPR-Cas technology to generate global and tissue-specific *gper* mutant zebrafish. Taken together, our data suggest that estrogens regulate heart rate via the G protein-coupled estrogen receptor.

451. Characterisation of Atrioventricular Valve Formation and its Regulation by *klf2a*. ***Emily Steed***, *Caroline Ramspacher, Stéphane Roth, Julien Vermot.* Department of Development and Stem Cells, IGBMC, Strasbourg, France.
The flow-responsive transcription factor *klf2a* has previously been implicated in cardiac valve formation, but the mechanism through which this is mediated is not yet known. Furthermore, the cellular processes involved in valve formation are relatively poorly understood. In light of this, we aimed to characterize endothelial cell behaviors during valve formation and to determine the role of *klf2a* during this developmental process. To begin, we performed three-dimensional analyses of the cellular arrangements within the atrioventricular canal (AVC) at the main stages of valve formation. Using transgenic lines in which the endothelial cell nuclei were fluorescently labeled, we saw that endothelial cells are unevenly distributed around the AVC at 48 hours post-fertilisation (hpf). Further cell clustering occurred by 56hpf and 72hpf, before the appearance of valve leaflets extending in to the AVC lumen. To determine the role of *klf2a* in this process, we generated a reporter line to observe the pattern of *klf2a* expression during valve development. Consistent with previous reports, we saw that *klf2a* expression is enriched in the AVC at 48hpf. Interestingly, we saw regional, cell-to-cell differences in klf2a expression levels within the AVC at 48hpf and are now investigating whether these differences correlate with the process of cell clustering that preceeds emanation of the atrioventricular valve. Using confocal microscopy and time-lapse imaging, combined with loss-of-function studies, we observed reduced cell clustering and impaired valve formation in the absence of *klf2a*. Together these data suggest atrioventricular valve formation occurs through a series of cell clustering events prior to valve emanation and that this process may be regulated by expression of *klf2a*. We are now combining in situ hybridization, qPCR and mRNA sequencing approaches to identify potential downstream targets of *klf2a* through which this behavior could be regulated.

452. Myocardium is Required for Endocardial Differentiation and Maintenance in Zebrafish. *Sharina Palencia Desai,* ***Saulius Sumanas***. Developmental Biology, Cincinnati Children's Hospital, Cincinnati, OH.
Endocardial and myocardial progenitors originate in distinct regions of the anterior lateral plate mesoderm and migrate to the midline where they coalesce into the cardiac tube. Endocardial progenitors acquire molecular identity distinct from other vascular endothelial cells and initiate expression of specific endocardial markers such as *nfatc1*. Yet the molecular pathways and tissue interactions involved in establishing specific endocardial identity are not well understood. We hypothesized that myocardium provides a signal required for endocardial differentiation. To test the role of myocardium in endocardial morphogenesis we used two different zebrafish models deficient in cardiomyocytes, the *hand2 (handsoff)* mutant and a myocardial-specific genetic ablation transgenic zebrafish line. Our analysis of myocardium-deficient hand2 mutants shows that early endocardial precursors migrate to the midline but fail to assemble into a cardiac cone and do not express markers of differentiated endocardium such as *nfatc*1. This phenotype can be rescued by myocardial but not endocardial specific expression of *hand2*. We then analyzed a genetic ablation model that specifically targets myocardial cells for death upon metronidazole (MTZ) treatment. In MTZ treated cmlc2:nitroreductase embryos apoptosis of myocardial cells is induced after 24 hpf, when the cardiac cone has already formed. This results in the progressive loss of *nfatc1* expression by 36 hpf. However endocardial cells are present and maintain their vascular endothelial identity as defined by kdrl expression until much later stages, even in the nearly complete absence of myocardium. We have further identified BMP as a candidate signal for endocardial differentiation. Chemical inhibition of BMP signaling at the tailbud stage resulted in severe inhibition of endocardial differentiation while there was little effect on myocardial development. Our results demonstrate that myocardium is critical for the morphogenesis and differentiation of endocardium as well as for its maintenance and identify BMP as a candidate signal involved in endocardial differentiation.

453. S1pr2/Ga13-mediated endoderm convergence controls the migration of endocardial migration. ***Huaping Xie***, *Ding Ye, Fang Lin.* Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, 1-400 Bowen Science Building, 51 Newton Road, Iowa city, IA 52242-1109, USA.
A key process in heart development is formation of the primitive heart tube, which has inner endocardia, and an outer myocardial, layer. This process depends on synchronized migration of the precursors of both layers, from lateral mesoderm to the midline, where each type forms a single population. We previously showed that S1pr2/Ga13-mediated convergent movement of the endoderm controls the medial

migration of myocardial precursors. However, the role of endoderm convergence in endocardia migration is not clear. Here we show that S1pr2/Ga13 signaling affects endocardia migration and the association between the myocardium and endocardium, resulting in two disorganized heart tubes in which the endocardium is misplaced as the outer layer. These phenotypes are rescued by endoderm-specific expression of a Ga13 transgene, indicating that endoderm convergence regulates endocardial migration. In vivo imaging revealed that during segmentation two populations of endocardial precursors initially migrated with the converging endoderm, but underwent subduction before completing their movement towards each other and merging at midline. The merged endocardial population then further traveled posteriorly towards myocardial primordia, forming the inner layer of the heart tube. In the context of defective endoderm convergence, the endocardial precursors failed to migrate towards the midline efficiently, moving towards the myocardial cells prematurely and positioning themselves at the dorsal region of that population. Mergence of endocardial and myocardial precursors resulted in hyper-epithelialization of the myocardial precursors; together with physical blockage of endocardial cells, this impaired further medial migration of the myocardial precursors. Notably, depletion of endocardial precursors partially rescued the defect in myocardial medial migration and the cardiac bifida. Together, our data indicate that endoderm convergence regulates the synchronized migration of endocardial and myocardial precursors, and demonstrate that endoderm movement plays a critical role in heart development.

454. Modeling a Rare Human Vascular Disease in Zebrafish. ***Jingwei R. Xiong***[1], *Arda Cetinkaya*[2], *A. Nurten Akarsu*[2], *Bruno Reversade*[1]. 1) Institute of Medical Biology, Singapore, Singapore; 2) Department of Medical Genetics, Hacettepe University Medical Faculty, Ankara, Turkey.
Vascular malformations are rare diseases which offer invaluable insights into the molecular basis of cardiovascular development and homeostasis. Here we describe the genetic and functional characteristics of an autosomal recessive vascular anomaly affecting the craniofacial region in humans. Patients present with initial jaw dysmorphisms followed by continuous intraosseous blood vessel expansion at the onset of puberty. Homozygosity mapping followed by whole exome sequencing delineated a critical locus on chromosome 20 containing the causative gene which encodes a protein involved in cell migration.
We have modeled the disease in zebrafish by morpholino-mediated depletion and CRISPR/Cas9-mediated knockout. Morphant and mutant embryos display severe jaw hypoplasia, blood vessel defects, pericardial edema, microcephaly and swim bladder defects. We noted a striking resemblance to zebrafish depleted of the histone demethylase *phf8*. Functional analyses show that Phf8 is a key upstream regulator of this gene which serves to mediate integrin signaling for vascular and craniofacial development in humans and zebrafish.

455. PDGF signaling regulates myocardial movement during heart tube assembly. *Joshua Bloomekatz, Ariel Dunn, Megan Vaughan,* ***Deborah Yelon***. Division of Biological Sciences, University of California, San Diego, La Jolla, CA.
Establishing the correct morphology of the heart requires the coordination of a complex series of cell movements. The initial steps of heart tube assembly involve the migration of myocardial and endocardial cells from bilateral positions to the midline, where they merge in a process called cardiac fusion. Despite prior studies that reveal a role for the adjacent endoderm in regulating cardiac fusion, the central question of which signals coordinate cardiac cell movement remains relatively unanswered. Here, we reveal a novel role for PDGF signaling in promoting cardiac fusion. We identified a zebrafish mutation called *refuse-to-fuse* (*ref*) that causes formation of a bifurcated, two-lobed ventricle connected to a dysmorphic atrial chamber. This aberrant morphology is the result of defective cardiac fusion: the *ref* mutation inhibits medial cardiomyocyte movement. Through positional cloning, we determined that the *ref* mutant phenotype is caused by disrupted splicing of the *pdgfra* gene, which is predicted to result in the premature truncation of the platelet-derived growth factor receptor alpha (Pdgfra). This result suggests a previously unappreciated function for PDGF signaling: although this pathway is known to influence later aspects of cardiogenesis, it has not been implicated in regulating cardiac fusion. Based on the expression patterns of *pdgfra* and the ligand-encoding gene *pdgfaa* in and near the heart fields, we propose a model in which PDGF signaling is one of the previously unknown pathways that coordinates the patterns of cell movement underlying cardiac fusion.

456. Investigating Proepicardial, Epicardial, and Myocardial Cells as Targets of TCDD Cardiotoxicity in Zebrafish Embryos. ***Monica S. Yue***[1], *Jessica S. Plavicki*[2], *Min-sik Kim*[2], *Peter Hofsteen*[2], *Richard E. Peterson*[1,2], *Warren Heideman*[1,2]. 1) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI; 2) School of Pharmacy, University of Wisconsin, Madison, WI.
Exposure to the ubiquitous environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes severe cardiovascular toxicity via activation of the AHR pathway. These effects are likely due to failure of the epicardium to form. The epicardium is the outer layer of the heart, and is important for heart development and regeneration. In the zebrafish heart the epicardium is derived from the proepicardium (PE), which forms adjacent to the heart and begins to migrate to the myocardium at 70 hours post fertilization (hpf). Recent work has shown that exposure to TCDD inhibits PE formation, epicardial progenitor cell migration, and the spread of epicardial cells across the myocardium. We hypothesize that the myocardium is a target site of TCDD that contributes to inhibition of PE migration and epicardial spread in TCDD-exposed hearts. To investigate this, we have activated AHR expression exclusively in myocardial cells using constitutively activated AHR (caAHR$^{ZF/M}$) linked to the *cardiac myosin light chain 2* (*cmlc2*) promoter. Here we show that caAHR$^{ZF/M}$ in myocardial cells does not inhibit development of the PE, however, the PE or PE cells fail to migrate onto the myocardium. This may explain the lack of epicardium formation in these caAHR$^{ZF/M}$ embryos. Similarly, *silent heart* morphants, which lack contractility, develop a PE but lack an epicardium. Additionally, using scanning electron micrography (SEM), we show that TCDD-exposed hearts have a smooth myocardial surface topography, which contrasts significantly from the rough surface of controls. To further examine the role of the myocardial cells on epicardium formation, we are developing an epicardial migration assay to assess the ability of untreated epicardial cells to spread onto the myocardium of TCDD-treated hearts *in vitro*, and to test whether caAHR$^{ZF/M}$ myocardial cells are receptive to migrating epicardial cells. (Supported by NIH ES012716 and 5T32ES007015-35).

457. Controlling the pace of cardiac differentiation: Cell adhesion molecule 4 restricts the production of outflow tract progenitor cells. ***Xin-Xin Zeng***, *Deborah Yelon.* Division of Biological Sciences, University of California-San Diego, La Jolla, CA.
Heart assembly requires input from two sources of progenitor cells - the first and second heart fields - that differentiate at distinct times and create different cardiac components. Notably, the cardiac outflow tract (OFT) is built through recruitment of second heart field (SHF)-derived cardiomyocytes (CMs) to the arterial pole of the heart. However, the mechanisms responsible for production of an appropriate number of OFT cells from the SHF remain unclear.
In zebrafish, we find that inhibition of Fgf signaling depletes OFT progenitors and impairs OFT formation, whereas increased Fgfr activity expands the OFT progenitor population. Additionally, we identify a gene, *cell adhesion molecule 4* (*cadm4*), that is negatively regulated by Fgf signaling and is expressed in a region near the developing arterial pole. Loss of *cadm4* function causes a dramatic enlargement of OFT: a surplus of SHF-derived progenitors aggregates around the arterial pole and ultimately results in the addition of nearly twice the normal number of OFT CMs. As is the case for the wild-type OFT, formation of this expanded OFT requires Fgf signaling. Conversely, increased expression of *cadm4* reduces the size of the OFT by decreasing the number of OFT progenitor cells. Furthermore, overexpression of *cadm4* overrides the ability of elevated Fgf signaling to enlarge the OFT progenitor population. Through cell-tracking analyses and EdU incorporation assays, we find a pool of OFT progenitors that reside in a relatively distal portion of the SHF and reveal that *cadm4* activity limits the proliferation of these progenitor cells prior to their deployment to the OFT. Altogether, our data support a model in which levels of *cadm4* activity, regulated by Fgf signaling, act to restrict the number of OFT progenitor cells that emerge from the SHF and the duration of their accumulation at the arterial pole. Our data are the first to suggest a role for cell adhesion in restraining SHF deployment to the OFT, perturbation of which could cause congenital OFT defects.

458. Automated high-throughput screen identifies drugs that induce insulin-producing beta-cell differentiation. ***Guangliang Wang***[1], *Surendra Rajpurohit*[4], *Fabien Delaspre*[1], *Sarah Townsley*[1], *Ruo-jing Li*[2], *Jun Liu*[2,3], *Jeffrey Mumm*[4], *Michael Parsons*[1]. 1) Department of Surgery, The Johns Hopkins University, Baltimore, MD; 2) Pharmacology and Molecular Sciences, The Johns Hopkins University, Baltimore, MD; 3) Department of Oncology, The Johns Hopkins University, Baltimore, MD; 4) Wilmer Eye Institute, The Johns Hopkins University, Baltimore, MD.
Type I diabetics (T1D) suffer from loss of insulin-producing b-cells in the pancreas leading to hyperglycemia and subsequent tissue damage. A significant step in developing b-cell replacement therapy is to identify compounds that stimulate the generation of new endogenous b cells. Using a strategy based on observation of endocrine islets in transgenic zebrafish larvae, we manually screened 3131 compounds to find compounds that induced b-cell differentiation. All screened compounds were from the Johns Hopkins Drug Library (JHDL), which consists of mainly clinically approved drugs. As such, this library is enriched for biologically active compounds with known targets. Due to the laborious nature of such a manual screen, only a single drug concentration was tested (10mM) and a very small number of replicates carried out. Although 6 FDA approved drugs were identified, we hypothesized that more compounds could be discovered, from the same library, if more concentrations were tested and on multiple sample replicates. To facilitate such a screen, we utilized the Automated Reporter Quantification In Vivo (ARQiv) system to streamline discovery of drugs that induce b-cell differentiation. With this high-throughput system, we performed a new drug screen for inducing insulin-producing islet formation. The screened drug doses ranged from 0.125 to 4 mM. We identified 24 drugs inducing endocrine islets from the JHDL. Next, we manually validated these hits and confirmed that 12 out of 24 drugs enhanced endocrine islet induction in zebrafish larvae. This screen and the hits we discovered has revealed novel pathways involved in b-cell differentiation. These new pathways represent potential targets that could be exploited in b-cell replacement therapy to treat T1D.

459. An In vivo Chemical Genetic Screen Identifies Phosphodiesterase 4 as a Novel Pharmacological Target for Hedgehog Inhibition. ***Charles H Williams***[1], *Jijun Hao*[3], *Audrey Frist*[2], *Jonathan Hemple*[1], *Jonathan Fleming*[1], *Gary Sulikowski*[1], *Michael Cooper*[1], *Chin Chiang*[1], *Charles C Hong*[1,2]. 1) Vanderbilt University, Nashville, TN; 2) Research Medicine, Veterans Affairs TVHS, Nashville, TN; 3) College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA.
Hedgehog (Hh) signaling plays an integral role in vertebrate development, and its dysregulation has been widely accepted as the driver of numerous malignancies. While a variety of small molecules target Smoothened (Smo) as a strategy for Hh inhibition, Smo gain of function mutations have limited their clinical implementation. Few details are known about the mechanism of Hh signaling downstream of Smo; however modulation of novel targets downstream of Smo could define a new paradigm for treatment of Hh-dependent cancers. Here, we describe eggmanone, a small molecule identified from a chemical genetic zebrafish screen which induced a Hh-null phenotype. Eggmanone exerts its Hh-inhibitory effects through selective antagonism of phosphodiesterase (PDE) 4 leading to protein kinase A (PKA) activation and subsequent Hh blockade. Our study implicates PDE4 as a novel target for Hh inhibition toward an improved strategy for Hh-dependent cancer therapy and identifies a unique probe of downstream of Smo Hh modulation.

460. Role of Wtip in the Chronic Kidney Disease. *R Powell, E Bubenshchikova, Y Fukuyo,* ***T Obara***. Cell Biology, OUHSC, Oklahoma City, OK, USA.
Chronic kidney disease (CKD) is characterized by progressive loss of function in the glomerular filtration barrier. CKD progresses to end-stage renal disease requiring dialysis or a kidney transplant. Podocyte foot processes and the slit diaphragm play a crucial role in development of a healthy glomerular filtration barrier. Changes in podocyte morphology are contemporaneous with the onset of proteinuria. Genes that are essential to podocyte function, such as WT1, have been identified, but the mechanism by which mutations in these genes cause glomerular filtration barrier failure is largely unknown. The zebrafish is an important vertebrate model for studying organogenesis and the cellular pathology underlying human diseases. Zebrafish pronephric podocytes undergo dynamic morphological changes analogous to mammalian metanephros development. During the development of the zebrafish glomerulus, the dorsal aorta invades to the immature podocyte cluster, initiating blood filtration at 40-48 hours post fertilization (hpf). At 96 hpf, the foot process and slit

diaphragm cell-cell junctions form to complete podocyte maturation. We demonstrated that depletion of Wtip, a basal body protein, or its interacting molecules (Wt1, Lats2, and Vangl2) results in early podocyte maturation at 48 hpf, compared with 96 hpf in wild type fish. The disruption in the timing of podocyte differentiation results leads to a failure of the glomerular filtration barrier. Furthermore, we have shown for the first time that 1) podocyte cilia are present in zebrafish, and 2) Wtip localizes to the basal body of podocyte cilia. We aim to understand how regulatory and signaling molecules function to establish a healthy glomerular filtration barrier, thereby providing a framework for developing interventions to restore lost kidney function.

461. Kinesins In Vertebrate Ciliogenesis. ***Niedharsan Pooranachandran****, Ulla-Maria Jokipii, Magdalena Widziolek, Jarema Malicki.* Biomedical science, University of Sheffield, Sheffield, United Kingdom.
Kinesins are motors that drive the transport of proteins necessary for both function and structure of cilia. Anterograde transport in vertebrate cilia has been proposed to require the presence of two heterotrimeric complexes consisting of Kif3a/Kif3b/Kap3 and Kif3a/Kif3c/Kap3. Previous work by our laboratory has shown that the simultaneous loss of Kif3b and Kif3c results in the absence of cilia in all tissues examined, although the function of Kif3c remains poorly investigated during late phases of ciliogenesis. To further investigate the function of Kif3c, we mutated the Kif3c gene in zebrafish and found that kif3c-/- embryos display normal embryonic development. In addition to the above, to test whether both kinesin 2 complexes require the Kif3a subunit, we are studying the ciliary phenotype of kif3a mutants. So far, we found that cilia are absent in all organs analyzed: nasal pit, spinal canal, the pronephric ducts, photoreceptors, and sensory patches of the ear by 5 days post fertilization. In parallel to this, we investigated additional motors, as they are considered necessary for the transport of specific functional components of the cilium. Thus we generated additional kinesin mutants in klp-6, and generated double mutants of different kinesin combinations. This analysis will enable us to characterize the repertoire of kinesins required by cilia of different organs and tissues. The outcomes of these studies will be reported.

462. Rsph9 depletion is associated with abnormal ciliary structure and aberrant neurulation. ***Jessica J. TeSlaa****, Abby Keller, Yevgenya Grinblat.* Zoology and Neuroscience, UW-Madison, Madison, WI.
Vertebrate embryos form the rudimentary central nervous system (neural tube) through the process of neurulation. Motile cilia are present in many fluid-filled organs during zebrafish development, including the spinal canal of the caudal neural tube. Here we present a characterization of these cilia in the ventral cranial neural tube, where distinct subpopulations of cilia reside in each subdivision of the brain primordium. By disrupting the radial spoke, a structural component of motile 9+2 cilia, we have also identified a novel role for motile cilia in the developing zebrafish neural tube. Radial spokes are multi-protein structures that link the outer microtubule doublets with the central microtubule pair in 9+2 cilia. Morpholino-mediated depletion of one radial spoke head component, Rsph9, results in embryos with overinflated ventricles. *rsph9* is expressed both in tissues that make 9+2 motile cilia, and in the ventral neural tube, which until now has been thought to produce only 9+0 cilia. Fluorescently labeled Rsph9 fusion protein localizes to the long cilia in the ventral cranial neural tube, pronephric ducts, and Kupffer's vesicle. Furthermore, we have shown by TEM that depletion of Rsph9 leads to abnormal formation of these cilia. These data support a role for motile cilia during early morphogenesis of the brain primordium and strongly suggest that the radial spoke is a critical component of cilia in the ventral neural tube of zebrafish embryos. We have generated a nonsense allele of *rsph9* using CRISPR mutagenesis, and will present a detailed characterization of ciliated organ morphogenesis in rsph9 mutant embryos.

463. Functional characterization of Prickle2 and BBS7 identify overlapping phenotypes yet distinct mechanisms. *Xue Mei,* ***Trudi A. Westfall****, Qihong Zhang, Val C. Sheffield, Alexander G. Bassuk, Diane C. Slusarski.* University of Iowa, Iowa City, IA.
Ciliopathies are genetic disorders that are caused by dysfunctional cilia and affect multiple organs. One type of ciliopathy, Bardet-Biedl Syndrome, is a rare disorder characterized by obesity, retinitis pigmentosa, polydactyly, mental retardation and susceptibility to cardiovascular diseases. The Wnt/Planar Cell Polarity (PCP) has been associated with cilia function and ciliogenesis in directing the orientation of cilia and basal bodies. Yet the exact relationship between PCP and ciliopathy is not well understood. We examined interactions between a core PCP component, *Prickle2* (Pk2), and a central BBS gene, *Bbs7*, using gene knockdown. *pk2* and *bbs7* knockdown both disrupt the formation the Kupffer's Vesicle (KV), but do not display a synergistic interaction. Using the neural tube as an assay for polarity, we find that *bbs7* activity is not required for pk asymmetric localization. We find that *pk2* knockdown suppresses *bbs7*-related retrograde melanosome transport delay. Similarly, knockdown of *ift22*, an anterograde intraflagellar transport component, also suppresses the *bbs7*-related retrograde delay. Notably, we find that *pk2* knockdown larvae show a delay in anterograde transport. These data suggest a novel role for Pk2 in directional intracellular transport and our analyses show that PCP and BBS function independently, yet result in overlapping phenotypes when knocked down in zebrafish.

464. Using the Zebrafish to Understand Developmental Control of DNA Replication. ***Joseph Siefert***[1,2]***, Chris Sansam***[1,2]. 1) Oklahoma Medical Research Foundation, Department of Cell Cycle and Cancer Biology, Oklahoma City, OK; 2) University of Oklahoma Health Science Center, Department of Cell Biology, Oklahoma City, OK.
In multicellular eukaryotes, DNA replication initiates at tens-of-thousands of origins spread throughout the genome. These origins do not fire all at once; instead small clusters of replication forks fire synchronously to replicate domains of sequence in a defined temporal order. These clusters of origins are also organized in the nucleus in a stereotypical spatial pattern that is conserved between cell types and organisms. While the majority of the genome replicates in the same order in most mammalian cell types, segments of the genome undergo early-to-late or late-to-early switches that correspond to transcriptional changes during embryonic development. The existence of the replication timing program has been known for over fifty years, yet we know little about how it is controlled and what it does. Oviparous organisms generally replicate their genome without a clear spatiotemporal pattern early in development and then establish replication timing with the onset of zygotic transcription. The zebrafish would serve as an ideal model system in which to study how the replication

timing program is established and the consequences of perturbing replication timing, but very little is known about replication timing in the fish. We will describe our progress in determining when replication timing changes occur during zebrafish development.

465. Retinoic acid independently and coordinately regulates size, pattern and alignment of neural and mesodermal tissues at the head-trunk transition. ***I. Skromne****, K. Lee.* Biology, University of Miami, Coral Gables, FL.
At the head-trunk transition, alignment of hindbrain and spinal cord territories to occipital and cervical structures is critical for coherent organization of neural and skeletal systems. Changes in neural or mesodermal tissue configuration arising from defects in territory size specification, patterning or relative axial placement can severely compromise system integration and function. Here we show that neural and mesodermal tissue coordination at the zebrafish head-trunk transition critically depends on two novel activities of the signaling factor Retinoic Acid (RA) specifying the size and axial position of the hindbrain territory relative to mesodermal structures. These activities are each independent but coordinated with RA's well-established function in hindbrain patterning. Using neural and mesodermal landmarks we demonstrate that RA function in aligning neural and mesodermal tissues temporally precedes the specification of hindbrain and spinal cord territories and the activation of hox transcription. Using cell transplantation assays we show that RA activity in the neuroepithelium is direct for hindbrain patterning but indirect for hindbrain territory size specification. This indirect function is independent of FGF and dependent on Wnts. Importantly, RA specifies and patterns the hindbrain territory by antagonizing the activity of the spinal cord specification gene cdx4; loss of Cdx4 rescues the defects associated with the loss of RA, including the reduction in hindbrain size and the loss of posterior rhombomeres. We propose that at the head-trunk transition, RA coordinates the specification, alignment and patterning of neural and mesodermal tissues essential for neural and skeletal system's functional organization.

466. Nanog Negatively Regulates Wnt/beta-catenin Pathway in Zebrafish. *Mu-Dan He, Feng-Hua Zhang, Hou-Peng Wang, Zuo-Yan Zhu,* ***Yong-Hua Sun***. State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, CAS, Wuhan, China.
Nanog encodes a homeobox containing transcription factor which is critical for the pluripotency and self-renewal of embryonic stem (ES) cells. In order to study the developmental role of *nanog*, we generated maternal-zygotic zebrafish mutant of *nanog* (*MZnanog*$^{-/-}$) through TALEN-mediated knock out. The *MZnanog*$^{-/-}$ mutants showed absence of *nanog* mRNA at both maternal and zygotic levels, and they could not normally pass the gastrulation. In previous study, it has shown that *nanog* regulates endoderm formation through the Mxtx2-mediated Nodal pathway. Therefore, *mxtx2* was overexpressed in *MZnanog-/-* mutants. Although *mxtx2* overexpression largely rescued the endoderm defects and the rescued embryos still developed with defects of anterior neuroectoderm, which resembled the phenotype after zygotic activation of Wnt/beta-catenin signaling. Further *in vivo* and *in vitro* studies revealed a novel role of zebrafish Nanog, which represses Wnt/beta-catenin transcription activity at protein-protein interacting level.

467. Distinct Signaling Roles for Type I Receptors Bmpr1 and Acvr1l, and the Type II Receptors Bmpr2 and Acvr2 within the BMP Receptor Complex. ***B. J. Tajer****, M. C. Mullins.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
The Bone Morphogenetic Protein (BMP) pathway patterns dorsal-ventral (DV) axial tissues during gastrulation. The zebrafish embryo is an excellent system to investigate the mechanism of BMP signal transduction during DV patterning, as many BMP pathway mutants are available and, unlike in mammals, these mutants survive to show DV patterning defects. When signaling, a dimeric BMP ligand assembles a receptor complex composed of two type I and two type II receptors. Type II receptors phosphorylate and activate type I receptors, which in turn phosphorylate Smad proteins. Phosphorylated Smad then regulates gene expression. This model, however, is overly simplistic as there are two conserved classes of type I receptor, Bmpr1 and Acvr1l, and two conserved classes of type II receptor Bmpr2 and Acvr2, all of which are necessary for vertebrate development. Our previous findings demonstrate that BMP2/7 heterodimers are the only ligands that signal in DV patterning. This sufficiency arises from the heterodimer's unique ability to integrate both type one receptors into the BMP receptor complex, as Bmpr1 preferentially binds the BMP2 ligand, and Acvr1l exclusively binds BMP7. Based on this and other data, I hypothesize that Acvr1l's necessity arises from its kinase domain, while Bmpr1's necessity arises from its ligand-binding domain. To test this hypothesis, I am performing a series of domain swap experiments to determine if Bmpr1's ligand binding domain and Acvr1's kinase domain are sufficient for DV patterning. We do not currently know the contribution of the two BMP type II receptor classes, Bmpr2 and Acvr2, to the signaling complex acting in DV patterning. While experiments in mice suggest that both classes are necessary for early embryonic development, these embryos die before DV patterning begins. I aim to create zebrafish mutants null for each entire type II receptor class, allowing us to determine whether both classes have independent, necessary signaling functions in DV patterning.

468. Rapid clearance of oscillating transcripts during somitogenesis requires the decay adapter Pnrc2 and spliceosome component Cdc5l/Cef1. ***Kiel T Tietz***[1]*, Thomas Gallagher*[1]*, Nicolas Derr*[1]*, Courtney French*[2]*, Jasmine McCammon*[2]*, Michael Goldrich*[2]*, Steven Brenner*[3]*, Sharon Amacher*[1]. 1) Department of Molecular Genetics, The Ohio State University, OH, USA; 2) Department of Molecular and Cell Biology, UC Berkeley, CA, USA; 3) Department of Plant and Microbial Biology, UC Berkeley, CA, USA.
Vertebrate segmentation is controlled by the segmentation clock, a biological oscillator that controls periodic formation of embryonic segments, or somites. This molecular oscillator generates cyclic gene expression in the presomitic mesoderm (PSM) and has the same periodicity as that of somite formation. Core cyclic components of the segmentation clock include the *hes/her* family of transcriptional repressors, but additional transcripts also cycle. Maintenance of the oscillation period requires that transcriptional activation and repression, RNA turnover, translation, and protein degradation are all very rapid. To investigate how cyclic genes are regulated during zebrafish development, we are using genetic and molecular approaches. We isolated two mutants, *tortuga*^{b644} and *honu*^{bk4}, in which cyclic transcript oscillations are abnormal; mutant embryos express ectopic mRNA in regions of the PSM where expression is normally off. Cyclic transcripts are differentially affected; some, like *her1*, are disrupted early during somitogenesis, whereas others, like *her7*, are not affected until several hours later. The mutants form fewer somites and display disorganized myofiber development. We have molecularly identified both *tortuga*^{b644} and *honu*^{bk4}. The *tortuga* gene encodes a Proline-rich nuclear receptor coactivator protein that has been implicated in

mRNA decay in cell culture systems, and we are investigating if Tortuga/Pnrc2 is part of a complex that rapidly degrades mRNA transcripts to maintain segmentation clock periodicity. The *honu* gene encodes a Cell division cycle 5-like protein that is a core component of the spliceosome in other systems, and we are currently investigating the relationship between RNA splicing efficiency and cyclic transcript turnover. Our goal is to define mechanisms critical for cyclic mRNA decay as we currently understand very little about the post-transcriptional control of oscillating genes during somitogenesis.

469. Mga is a novel regulator of neural crest development. ***Wei-Chia Tseng***, *Scott Dougan.* Department of Cellular Biology, University of Georgia, Athens, GA.
Max-gene associated (Mga) is a member of T-box6/Spadetail transcription factor family and has both T-box domain and bHLHzip domain for DNA binding. It is ubiquitously expressed both maternally and zygotically until the end of gastrulation, suggesting its possible importance in early development. It is known that Mga promotes exiting of cell cycle by antagonizing Myc activity. Previous study shows that Mga is important in development of brain, heart and gut in zebrafish. Mga also regulates Bmp expression in the extra-embryonic yolk syncytial layer (YSL) and contributes to dorsoventral pattern formation. Here we demonstrate that Mga plays a novel role to promote in neural crest (NC) development. During embryonic development in vertebrates, NC cells are a group of multipotent cells that will give rise to a variety of tissues, such as pigment cells, craniofacial cartilages and peripheral nervous system. NC development is tightly controlled under several signaling pathways including Bmp and Wnt signaling at different stages, but its spatial and temporal regulations are not well understood. In addition to the defects described in previous study, depletion of Mga by morpholino oligonucleotides leads to general NC defects such as malformations of craniofacial and pharyngeal cartilages, reduced pigmentation on the skin and defective enteric neurons. More apoptotic cells are found in migratory NC population in Mga morphants. These embryos also exhibit reduced expression level of early NC marker genes including Sox10, Tfap2a, Snai1b and Foxd3, suggesting Mga is involved in early NC specification. Additionally, Bmp signaling is reduced in Mga morphants before NC cells formed. Therefore, we hypothesize that Mga contributes to proliferation and/or differentiation by regulating Bmp signaling or by antagonizing the function of Myc. We are still testing the hypothesis in order to understand the correlation between Mga and NC development.

470. Shaping the BMP signaling gradient through the regulation of metalloprotease activity. ***Francesca Tuazon***[1], *Joseph Zinski*[1], *Amy Kugath*[1], *Yan Huang*[2], *Wei Dou*[2], *David Umulis*[2], *Mary Mullins*[1]. 1) University of Pennsylvania, Philadelphia, PA; 2) Purdue University, West Lafayette, IN.
Bone Morphogenetic Proteins (BMPs) act in a morphogen gradient to pattern the dorsoventral (DV) axis in invertebrates and vertebrates. The shape of the BMP gradient across the DV axis specifies distinct cell fates in precise domains. In the zebrafish embryo, high BMP signaling specifies ventral cell fates, intermediate levels specify lateral fates, and no BMP signaling results in dorsal fates. At the mid-blastula stage, the initial BMP signaling gradient is generated by Chordin (Chd), a key extracellular antagonist that binds BMPs to inhibit signaling. Chd itself is regulated by two key classes of proteins: (i) the highly homologous metalloproteases Tolloid (Tld) and Bmp1a, which cleave and inactivate Chd, and (ii) the metalloprotease inhibitor Sizzled (Szl). We found that MZ-bmp1a mutants do not have defects in DV patterning, indicating that Bmp1a is not essential for DV patterning. However, tld;bmp1a double mutants exhibit a moderately dorsalized phenotype, which indicates that these metalloproteases function redundantly and is consistent with previous morpholino knockdown studies. We have determined the shape of the BMP signaling gradient through a quantitative immunofluorescence assay of nuclear phosphorylated Smad1/5, a direct readout of active BMP signaling. We have quantified the shape of the wild-type BMP signaling gradient between early- and late gastrulation stages and found that it steepens dramatically and rapidly between these stages. The altered BMP signaling gradient in tld, tld;bmp1a, and szl mutants indicates that regulation of metalloprotease activity is absolutely required to correctly shape the BMP gradient during trunk and tail patterning. Each mutant displays unique changes in the shape of the BMP gradient, suggesting distinct region-specific roles for Tld/Bmp1a, and Szl. These data support a novel requirement for metalloprotease regulation to correctly steepen the BMP signaling gradient from mid- to late gastrulation stages to pattern the posterior tissues of the zebrafish embryo.

471. Using single molecule RNA *in situ* hybridization to analyze the establishment of gene expression patterns during zebrafish embryogenesis. *L. Carine Stapel*, ***Nadine L. Vastenhouw***. MPI-CBG, Dresden, Germany.
Transcription is thought to be a stochastic process, resulting in significant variation in gene expression between cells in a population. While variation in gene expression levels may be important in some cases, many developmental processes depend upon uniform expression of patterning genes. Here, we used single molecule RNA *in situ* hybridization to quantify gene expression levels during zebrafish embryogenesis. We found that at sphere stage, all genes we analyzed (n=14; including housekeeping genes and developmental regulators) are generally expressed to similar levels in all cells (within their expression domain). To determine how this homogeneity is achieved during development, we analyzed the pattern of gene expression for these genes from the moment they are first transcribed. We show that for some genes, the uniform expression pattern is established immediately when transcription starts while for other genes, we initially observe significant variation in expression between cells. This suggests that the uniform expression pattern observed at later stages can be established immediately or recovered over time. We are currently analyzing the differences between the different routes to homogeneity.

472. Essential role of chromatin remodeling protein Bptf in the induction and patterning of the neuroectoderm. *Yuanqin Ma, Guozhu Ning, Yu Cao*, ***Qiang Wang***. Institute of Zoology, CAS, Beijing, Beijing, China.
During vertebrate gastrulation, “neuralizing” signal from the organizer induces anterior neural tissue and “posteriorizing” signal from the ventral mesoderm converts already neuralized tissue to more posterior neural tissues. Here, we show that, in zebrafish embryos, the chromatin remodeling protein bptf plays crucial roles during the induction and patterning of the anterior vs. the posterior neuroectoderm. Bptf functionally and physically interacts with Smad2. Bptf and Nodal/Smad2 co-regulate a major Bmp antagonist chordin expression to promote neural induction at the onset of gastrulation. We also find that Bptf acts cooperatively with Smad2 in neural posteriorization at the

late gastrula stage, although Nodal signal deficient Embryos have appropriate anteroposterior patterning. Furthermore, we discover that TGF-b signal functions in transforming anterior neural tissue to more posterior identity via Bptf and Smad2 regulated wnt8a expression. Thus, Bptf promotes the induction and patterning of the neuroectoderm as a co-regulatory factor of Smad2 under different TGF-b super family signals during early zebrafish embryogenesis.

473. *ptf1a* lineage allocation in zebrafish pancreas. ***Yue. J Wang***, *Michael. J. Parsons, Steven. D. Leach.* Institute of Genetic Medicine, Johns Hopkins, Baltimore, MD.
The vertebrate pancreas develops from dorsal and ventral buds in the foregut endoderm. The expression of a basic-helix-loop-helix (bHLH) transcription factor---*ptf1a*, in the pancreatic progenitor cells, commits these cells to dedicated pancreatic lineage. In zebrafish, during early embryogenesis (0-72 hour post fertilization (hpf)), the level of Ptf1a activity influences the fate of pancreatic cells: high levels of Ptf1a activity promote exocrine cell fates, whereas low levels of Ptf1a activity are compatible with endocrine differentiation. Our lab recently generated a *ptf1a:creERT2* BAC transgenic fish line. This line has been crossed onto *ubi:loxp-CFP-loxp-nuc-mCherry* reporter fish. The resulting *ptf1a* lineage-tracing fish (*ptf1a* LT) is under control of both *ptf1a* expression and tamoxifen availability. Upon the application of tamoxifen, cells with cre activity will be permanently labeled by nuclear mCherry. We compared the *ptf1a* lineage allocation in normal pancreas and in the pancreas of ptf1a heterozygous fish (sa126). We showed that in normal pancreas, the majority of *ptf1a* lineage labelled cells contribute to exocrine. Interestingly, in sa126 fish, the fate of *ptf1a* lineage labelled cells become less restricted; a bigger proportion of cells can be traced into pancreatic Notch-responsive-cell (PNC) compartment. Currently, we are carrying out experiment to further clarity the interplay of Ptf1a and Notch singling pathway during zebrafish pancreas development. Insight we get from this study will potentially guide us in cell engineering *in vitro*.

474. Global identification of Eomesodermin a targets in zebrafish identifies a novel role for Eomesodermin in repression of ectodermal gene expression. *Andrew C Nelson[1,5], Stephen J Cutty[1], Derek L Stemple[2], Paul Flicek[3], Ashley EE Bruce[4],* ***Fiona C Wardle***[1]. 1) Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom; 2) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1HH, UK; 3) European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD; 4) Department of Cell and Systems Biology, University of Toronto, 25 Harbord Street, Toronto, ON Canada M5S 3G5; 5) Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE, UK.
In zebrafish the T-box family transcription factor Eomesodermin a (Eomesa) plays an important role in early endoderm and mesoderm formation and patterning. In order to identify the downstream targets through which this factor acts, we have used ChIP-seq to map genomic binding of Eomesa at blastula stages. We show that Eomesa acts in concert with Smad2 to up-regulate conserved mesoderm and endodermal target genes, but unexpectedly we find that Eomesa also represses ectodermal gene expression in the early blastula embryo.

475. Zebrafish Tbx16 regulates intermediate mesoderm cell fate by attenuating Fgf activity. ***Rachel M. Warga***[1,3], *Rachel L. Mueller*[2,3], *Robert K. Ho*[3], *Donald A. Kane*[1]. 1) Biological Sciences, Western Michigan University, Kalamazoo, MI; 2) Department of Biology, Colorado State University, Fort Collins CO; 3) Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL.
Progenitors of the zebrafish pronephros, red blood and trunk endothelium all originate from the ventral mesoderm and often share lineage with one another, suggesting that their initial patterning is linked. Previous studies have shown that spadetail (spt) mutant embryos, defective in tbx16 gene function, fail to produce red blood cells, but retain the normal number of endothelial and pronephric cells. We report here that spt mutants are deficient in all the types of early blood, have fewer endothelial cells as well as far more pronephric cells compared to wildtype. In vivo cell tracing experiments reveal that blood and endothelium originate in spt mutants almost exclusively from the dorsal mesoderm whereas, pronephros and tail originate from both dorsal and ventral mesoderm. Together these findings suggest possible defects in posterior patterning. In accord with this, gene expression analysis show that mesodermal derivatives within the trunk and tail of spt mutants have acquired more posterior identity. Secreted signaling molecules belonging to the Fgf, Wnt and Bmp families have been implicated as patterning factors of the posterior mesoderm. Further investigation demonstrate that Fgf and Wnt signaling are elevated throughout the nonaxial region of the spt gastrula. By manipulating Fgf8a signaling by heatshock overexpression, antisense morpholino knockdown and SU5402, a chemical antagonist of the Fgf receptor we show that Fgfs both promote pronephric fate and repress blood and endothelial fate. We conclude that Tbx16 plays an important role in regulating the balance of intermediate mesoderm fates by attenuating Fgf activity.

476. The Role of BMP signaling in fissure closure in the vertebrate eye. ***Sonya A Widen***, *Prajakta Desai, Curtis R French, Ordan Lehmann, Andrew Waskiewicz.* University of Alberta, Edmonton, Canada.
Proper development of the vertebrate embryo requires fusion of epithelial cell sheets, resulting in closure of the developing neural tube, palate and retina. Within the eye, failure of optic fissure closure results in ocular coloboma, a leading cause of pediatric blindness. Previous work from our laboratory has defined a key role for Bone Morphogenetic Protein (BMP) signaling in regulating optic fissure closure. BMP mRNAs are expressed in the dorsal retina and regulate phosphorylation of Smad effectors in this region. Outside of the retina, a population of neural crest cells known as periocular mesenchyme (POM) migrates to the fissure sites and is required for fissure closure in the eye, although the mechanism is currently unknown. Here we show data identifying a novel regulator of ocular fissure closure: bmp3. Sequencing of 480 patients with coloboma has identified five with variants in BMP3. Such mutations (A188D, K345N, S393F, F450Y, and A470F) are in conserved residues with four of five affecting the ligand domain and predicted by Polyphen to damage protein function. Absence of such variants in exome databases and control samples further demonstrates the likely pathogenicity of these mutations. Studies in cultured cells demonstrate that the Bmp3 variants have altered activity in vitro. In zebrafish, loss of bmp3 results in fissure closure defects, implicating this ligand as a key regulator of eye morphogenesis. Surprisingly the bmp3 transcript is expressed not in ocular tissue,

but in the migratory POM cells. We hypothesize that bmp3 regulates POM migration or signaling between POM and retina. Current studies will address whether the identified variants affect biological activity when expressed in zebrafish. We have begun injecting targeted nucleases (TALENs) to generate a zebrafish mutant lacking bmp3 as a model to define the molecular mechanisms underlying POM function in optic fissure closure. We are also attempting to identify the downstream effectors of Bmp3 using RNA-Seq. Our research will define the role of BMP3 in ocular development and help to define the molecular mechanisms underlying POM-retina signaling in the process of optic fissure closure.

477. Identification and embryonic expression pattern of a highly conserved *Meis*-linked gene and its protein product. ***Zach Williams***[1], *W. Tray Neilson*[1], *Brandon S. Carpenter*[2], *A. Caroline Cochrane*[3], *Brantley Graham*[4], *Ted Zerucha*[1]. 1) Biology, Appalachian State University, Boone, NC; 2) University of Michigan Medical School Program in Biomedical Sciences, Ann Arbor, MI, USA; 3) University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA; 4) University of Kentucky Spinal Cord and Brain Injury Research Center, Lexington KY, USA.
We have identified a novel and previously uncharacterized gene, zgc:154061 that is located directly downstream of the zebrafish meis2.2 gene. Putative orthologs of this gene have been identified in all animals for which publicly available genome data is available and it is always found directly downstream of Meis2. The zgc:154061 gene and its vertebrate orthologs are organized in a convergently transcribed manner with respect to the Meis2 gene in all species we have examined (meis2.2 in teleosts). During zebrafish development, transcripts of zgc:154061 are observed in every cell of the embryo from the earliest stage through the shield stage. Expression of zgc:154061 gradually decreases from its peak value at 0 hpf until 8 hpf and then is observed to be activated again at 12 hpf as determined by quantitative real time PCR. This later expression is observed throughout the neural tube before becoming restricted to the retina and tectum opticum by 48 hpf. Whole mount and cross-section immunohistochemistry, using an antibody raised against a peptide portion of the predicted zebrafish protein product, has revealed that the gene is actively translated into protein and highly localized to the retinal area and optic nerve. Western blots have shown the protein to be expressed during all stages of development and as early as 2hpf.

478. Wnt5a is Crucial for Kidney Development. ***An Xiao***[1,2], *Andrea Wecker*[1], *Weibin Zhou*[3], *Bo Zhang*[2], *Joshua H. Lipschutz*[4], *Liwei Huang*[1,4]. 1) Eastern Virginia Medical School, Norfolk, VA; 2) Peking University, Beijing, China; 3) University of Michigan, Ann Arbor, MI; 4) University of Pennsylvania, Philadelphia, PA.
Polycystic kidney disease is one of the leading causes of end-stage renal disease in humans and is characterized by progressive cyst formation, renal enlargement, and abnormal tubular development. Wnt5a is a non-canonical secreted glycoprotein of the Wnt family that plays an important role in organ development. Wnt5a missense mutations are associated with Robinow syndrome, a genetic disorder affecting many organs, including abnormalities in the urinary system. Cystic kidney disease was reported in patients with this syndrome. However, little is known regarding Wnt5a and kidney cyst formation. As we previously reported, knockdown of wnt5a in zebrafish by antisense morpholinos resulted in cyst formation in glomerula and proximal renal tubules, which were visualized in the background of a transgenic zebrafish line with the pronephros marked by fluorescence proteins. These phenotypes can be partially rescued by co-injection of mouse Wnt5a mRNA, suggesting they are specifically caused by disruption of wnt5a. However, morpholino-knockdown has many disadvantages in zebrafish research. The phenotypes are temporary, not heritable, and may be caused by side-effects. Full knockout of wnt5a is needed to build a cystic kidney disease model for further study of the mechanism of cyst formation in zebrafish pronephros. The CRISPR/Cas system is a newly developed technology of engineered endonuclease for targeted cleavage and manipulation of genomes. It can be applied to various species, including zebrafish, in which gene knockouts were previously impossible. With these customized endonucleases, we successfully targeted dozens of zebrafish genes by different approaches. We have now designed specific CRISPR/Cas systems targeting the second and fourth exons of wnt5a. This will fully disrupt the gene at the DNA level by randomly inducing small indels in the coding sequences or major deletions in the gene. Our previous data indicated that wnt5a was centrally involved in kidney development.The wnt5a knockout zebrafish model will enable to more fully investigate the role of wnt5a in pronephro cyst formation.

479. Microfluidic chorionic fluid extraction for genotyping zebrafish embryos. ***Raheel Samuel***[1], *Regan Stephenson*[2], *Paula Roy*[2], *Rob Pryor*[3], *Luming Zhou*[3], *Joshua Bonkowsky*[2], *Bruce Gale*[1]. 1) Department of Mechanical Engineering, University of Utah, Salt Lake CIty, USA; 2) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, USA; 3) Department of Pathology, University of Utah School of Medicine, Salt Lake City, USA.
We introduce two novel methods for genotyping zebrafish embryos: viability, morphology, behavior, and overall health of the embryos is preserved. In contrast, there is no available technology for embryonic genotyping without sacrificing the animals. Thus genotyping, genetic screens, or drugs/therapeutics trials cannot be performed on animals of known genotype until 4-8 weeks of age. Our first method utilizes microfluidic technology to extract embryonic chorionic fluid at 24hpf, followed by DNA extraction from sloughed cells in the chorionic fluid, PCR amplification, and high-resolution melt analysis. Our second method is performed at ~35 hpf, using a modified fin clip, in which the embryo is held in a channel and a PDMS membrane is lowered to amputate the fin distal to the caudal blood artery. For both methods the embryo is kept alive in a separate well until PCR analysis is complete. We tested both approaches: embryos were observed for 5 days post-analysis and had 100% viability. All larva had normal morphology and touch response at 72 hpf. Fin regeneration was seen by 7 dpf for embryos with the fin clip method. Sensitivity for genotyping was 78% for the chorionic fluid method and 100% for the fin clip method. To demonstrate that the genetic material tested in chorionic fluid is embryonic, non-maternal source, we used a cross of wild-type adult male fish to females heterozygous for a Gal4-transgene. Embryos are either wild-type (GFP-negative), or Gal4+ (GFP-positive). Embryos were manually sorted based on the visual presence of the cardiac GFP at 24hpf. We found that all of the wild-type embryos had no PCR amplification, demonstrating that no maternal cells or DNA were amplified from the chorionic fluid. Conversely, all transgenic embryos had successful PCR for Gal4. Additional development can scale these novel genotyping methods for high-throughput screening, which would complement the cost-effectiveness of using zebrafish disease models.

480. Rapid Identification and Recovery of ENU-induced Mutations in High-Priority Genes with MiSeq Paired-End Low-Error Sequencing.
*Luyuan Pan[1], **Arish Shah**[1], Ian Phelps[2], Dan Doherty[2], Eric A. Johnson[3], Anna Hindes[4], Lila Solnica-Krezel[4], Cecilia B. Moens[1]*. 1) Dvision of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, Washington, USA; 3) Institute of Molecular Biology, University of Oregon, Eugene, Oregon, USA; 4) Department of Developmental Biology, Washington University School of Medicine, St. Louis, Missouri, USA.
Targeting Induced Local Lesions IN Genomes (TILLING) is a reverse genetics approach to identify rare point mutations in specific genes of interest in large chemically mutagenized populations. Classical TILLING, based on enzymatic detection of mutations in heteroduplex PCR amplicons, is slow and labor intensive. Here we describe a new TILLING strategy in zebrafish using direct next generation sequencing of 250 bp amplicons with Paired End Low-Error (PELE) sequence analysis. This new TILLING protocol significantly increases screening efficiency and accuracy at reduced cost and can be applied in a wide range of organisms. By pooling the library in 32 equal pools of 288 fish each with a separate Illumina index, we reduce the complexity of the template to a level at which we can detect mutations that occur in only a single heterozygous fish in the entire library of almost 10,000 cryopreserved F1 ENU-mutagenized fish. Screening a total of 5.5 kb (22 ~250 bp fragments) in a single MiSeq lane, we identify an average of 6 nonsense mutations. This predicts an 85% chance that a nonsense mutation can be found in our library in any gene with at least 1kb coding sequence. Importantly, the time from fragment design to generation of F2 heterozygotes is 4-6 weeks compared to six months to generation of F2 heterozygotes using TALENs or CRISPR. We encourage submissions of target genes at http://webapps.fhcrc.org/science/tilling/index.php. This work is supported by NIH Grant HD076585 to C.B.M. and L.S.K.

481. In-vivo Protein Trap for Functional Annotation of Zebrafish Genome.
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Gene breaking trap (GBT) vector is an efficient forward genetic tool to trap, disrupt and identify novel genes to explore their expression and sub cellular localization. GBT vectors are built to mimic the endogenous gene structure and are composed of two cassettes viz; mutagenesity cassette and the gene trapping cassette which facilitates identification of native transcripts. This system is completely unbiased towards the location and abundance of the endogenous transcripts, therefore can trap and mutate the coding as well as the non-coding repertoire of the genome that would not be isolated in a classical forward genetics screens. The advantageous feature of the GBT vectors is that, they create conditional mutants whereby the mutant alleles can be reverted in somatic tissues via Cre recombinase or splice-site-blocking morpholinos. The RP2, an in-vivo protein trap system, is capable of efficiently disrupting gene expression and simultaneously report the protein expression for the disrupted loci. To our knowledge this is the first systematic conditional mutant alleles in vertebrates outside the mouse model. With an aim to functionally annotate the zebrafish genome with phenotypically observable tissue specific expression pattern, we have initiated an insertional mutagenesis screen in zebrafish using the RP2 gene breaking trap method. Till date we have screened 595 individual fish and identified 60 transgenics with diferrent expression patterns across 9 different tissues types. Currently, we are utilizing a next generation sequencing method to identify the trapped loci and then systematically use them for mutational screening and hope to link tissue specific expression with precise function in the respective tissues. Acknowledging the high degree of functional conservation between zebrafish and human genome, we hope this functional annotation of the zebrafish genome would help us to model and better understand the human disease biology.

482. Zebrafish ENCODE: The Next Big Milestone in Genomic Research of Zebrafish.
*Shadabul Haque, Vinod Scaria, **Sridhar Sivasubbu***. CSIR Institute of Genomics and Integrative Biology, Delhi, Delhi, India.
In recent years the human genome project and the human ENCODE project have significantly added to our understanding of human genomic and epigenomic landmarks. Similar noteworthy projects have been initiated for other model systems such as the mouse ENCODE and the modENCODE for C. elegans and Drosophila. However, international collaborative efforts towards constructing a comprehensive encyclopaedia of functionally annotated regulatory elements in the Zebrafish genome are still in the infancy. Our current understanding of the Zebrafish genomics together with the latest technological advancement and the presence of standardized protocols provides the right cocktail for the functional annotation of Zebrafish genome. Recently the availability of Zebrafish reference genome together with the sequences of multiple strains including the wild Zebrafish genome have provided a much needed base for understanding the dynamics of genome organization and initiating its functional annotation activity. Lately deep sequencing of genome at different development time-points and in varied adult tissues types have helped us to understand the regulatory transcriptome of Zebrafish. In addition, the Zebrafish genome engineering technologies such as transposon-based gene trapping, morpholinos antisense oligonucleotide based knock-down, transcription activator like effector nucleases based targeted insertion/deletion provides tools for the interrogation of gene function at a whole organism level. These tools have enabled the Zebrafish community to generate picturesque insights of Zebrafish genomic landscape as well as its dynamic expression patterns. These favourable circumstances warrants for the creation of Zebrafish ENCODE for collaborative concerted efforts towards extracting functional information from the Zebrafish genome. Unlike the directed top-down initiative like modENCODE and mouse ENCODE the Zebrafish community could adopt an alternative approach involving and open collaboration and open access strategies through a ‘bottom-up’ approach where the research labs are free to generate and publish the data, however the integrated data is processed through a standard common pipeline and made available to all participants.

483. Genome-wide Knockdowns to Study Hemostasis in Zebrafish by Using Deoxyoligonucleotides Piggybacked with Vivo-Morpholinos.
*G. Khandekar, **H. Sundaramoorthi**, P. Jagadeeswaran.* Biological Sciences, University of North Texas, Denton, TX.
Hemostasis is a defense mechanism that evolved to prevent loss of blood in the event of an injury. If this same process occurs inside the blood vessel and continues unchecked, it results in occlusion of the vessel, or thrombosis. Both hemostasis and thrombosis are regulated by

three components: the vessel wall, the components of the vessel, and the factors that determine the blood flow. Despite a century's worth of intensive research, only approximately two hundred factors have been identified. These include blood clotting factors, platelet receptors and their downstream signaling pathway proteins, and endothelial cell factors; yet more factors remain to be discovered. To comprehensively identify most of these unknown factors, we introduced the zebrafish model almost two decades ago to study the genetics of hemostasis. Using the classical forward genetic approach with ENU mutagenesis and positional cloning methods, we successfully isolated a novel *GPR34l* mutant in the hemostatic pathway. However, progress using this approach has been frustratingly slow. Therefore, we designed a novel, cost-effective knockdown method and started genome-wide knockdowns to study hemostasis in adult zebrafish. This method uses a unique Vivo-Morpholino design, the piggyback Vivo-Morpholino. The piggyback Vivo-Morpholino is a hybrid molecule made of a non-gene Vivo-Morpholino and a gene-specific standard oligonucleotide. To validate use of this hybrid molecule in gene knockdowns, we targeted the thrombocyte specific *itga2b* gene. The use of this piggyback technology resulted in thrombocyte functional defects comparable to those obtained with standard Vivo-Morpholino knockdown and is considerably less expensive. At present we have performed 2000 gene knockdowns and have identified more than 50 genes involved in hemostatic function. Thus, identification of most of the genes in hemostatic pathways by these genome-wide knockdowns is possible and will provide a foundation for future work on novel therapeutic targets to treat hemostatic and thrombotic defects.

484. Transcriptomic signature of mature endocrine pancreatic cells. ***E. Tarifeno-Saldivia**, A. Lavergne, K. Padamata, I. Manfroid, M. Voz, B. Peers.* Laboratory of Zebrafish Development and Disease Models, GIGA-R, University of Liege, Belgium.
Pancreas is a mixed gland composed of endocrine and exocrine tissues and plays a crucial role in the metabolism of all vertebrates. The endocrine cells are mainly grouped into the islets of Langerhans and secrete distinct hormones, such as glucagon (a-cell), insulin (b-cell), somatostastin (d-cell) and ghrelin (e- cell). Diabetes occurs when insulin production by the b-cells is unable to counteract increase of glycemia. Better knowledge on pancreatic cell differentiation, regeneration and physiology is needed to design novel therapies for diabetes. While several transcription factors have been identified as pivotal for the differentiation of the various pancreatic cell types, there is still no comprehensive list of genes presenting cell type-specific expression. In a first step, we have determined the transcriptional landscape of the zebrafish mature alpha, beta and delta endocrine cells as well as exocrine cells. This was achieved by dissecting pancreas from the transgenic insulin:GFP, glucagon:GFP, somatostatin:GFP and ptf1a:GFP lines, dissociation and sorting GFP cells by FACS, RNA extraction, synthesis of cDNA and sequencing on Illumina platform. Experiments were performed in duplicates for each cell type and about 40 millions of sequenced reads were obtained per sample. Sequences were aligned to the zebrafish genome and expression level per-gene was measured using HTSeq software. Heatmap plot and principal component analysis show that samples belonging to the same cell type cluster together, indicating that transcriptome profiles are characteristics to different cell types. Cell type specific transcripts were identified using the DEseq and EBSeq software. Among these genes are transcription factors known to control pancreatic cell differentiation (i.e. pdx1, nkx6.2, ptf1a). Some new cell type specific transcripts were selected based on their GO annotation and studied by ISH. In order to define a common vertebrate signature, comparative transcriptomics for endocrine and exocrine tissue were performed using previous published data. This cross-species comparison should highlight genes having an evolutionary conserved action distinguishing them as important for the pancreas physiology.

485. Large-scale Targeted Knockout Production in Zebrafish. ***Richard J. White**, Ian M. Sealy, Alex Hodgkins, Vivek Iyer, Isobel Brocal, Elisabeth M. Busch-Nentwich, Christopher M. Dooley, Derek L. Stemple, Ross N. W. Kettleborough.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA. United Kingdom.
The CRISPR/Cas9 endonuclease system is now widely used in zebrafish for genome editing due to the ease with which the reagents required can be produced. As part of the Zebrafish Mutation Project (ZMP), we have been developing a system for routinely generating CRISPR/Cas9 knockouts at high throughput to complement our existing ENU mutagenesis strategy. To design large numbers of CRISPR guide RNAs, we have developed software to analyze all possible CRISPR/Cas9 target sites within a particular region and select the best candidates. The two main criteria are the position of the site within the target region and the potential for off-target effects. We use next-generation sequence mapping methods to assess all possible off-target sites for a given candidate CRISPR target site and select those with the lowest possible off-target potential. To engineer larger deletions we design pairs of guide RNAs, which requires analysis of the off-target potential of all possible combinations of CRISPR target sites. An interval tree allows us to efficiently compute all possible pairs of off-target sites that lie within a specified distance of one another. To facilitate screening large numbers of mosaic G0 fish we have developed an amplicon-based Illumina sequencing protocol. A two-step PCR process is used to produce barcoded Illumina libraries ready for sequencing for a specific set of target amplicons. We can use this method on both injected G0 fish and their offspring to quickly and easily assess both the efficiency and the germline transmission rate for multiple different CRISPR guide RNAs. We intend to use this high-throughput method to generate knockouts in genes for which we have not currently isolated ENU alleles to help us reach our goal of generating a knockout in every protein-coding gene in the zebrafish genome.

486. Population Genomics Reveals Strain-Specific Sex Determination Loci in the Zebrafish. ***Catherine Wilson**[1], Samantha High[1], Angel Amores[1], Peter Batzel[1], Yilin Yan[1], Tom Titus[1], Jennifer Anderson[1], Manfred Schartl[2], John Postlethwait[1].* 1) Institute of Neuroscience, University of Oregon, Eugene, OR; 2) University of Würzburg, Würburg, Germany.
Mechanisms of sex determination in fish are diverse and can vary even among closely related species. The zebrafish (*Danio rerio*) lacks heteromorphic sex chromosomes, and their mechanism of sex determination remains unknown. Environmental factors such as nutrition, density and temperature can influence sex ratios, but multiple studies suggest that there is a genetic component to sex determination as well. Recent experiments using F2 mapping crosses to map sex-associated loci suggest that zebrafish has a polygenic sex determination system, and that different loci may be involved in different strains. To resolve conflicting results from mapping studies, we conducted a population genomic analysis of restriction site-associated DNA markers (RAD-tags) to identify sex-associated SNPs in 334 fish from six

different wild-type zebrafish strains. A genome scan for sex-associated loci identified strong linkage on the end of the heterochromatic long arm of chromosome 4 in the recently derived wild-type strains Nadia, WIK, and Ekkwill, as well as a wild strain obtained from the Cooch Behar region of India. Remarkably, we were unable to identify any significant genetic contribution to sex in both AB and Tuebingen strains, suggesting that the major sex determinant has been lost in the most commonly used laboratory strains. Our data suggest that chromosome 4 is a homomorphic WZ sex chromosome in wild populations of zebrafish that has been lost during the domestication of laboratory strains.

487. GRCz10: An Improved Reference Genome Assembly. ***Jonathan Wood****, Katherine Auger, Kerstin Howe, on behalf of the Genome Reference Consortium.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom.
Since the release of the current Zebrafish Genome Assembly, Zv9, in 2010, the Genome Reference Consortium (GRC, www.genomereference.org) has been working towards improving the clone path to correct misrepresented regions and fill remaining gaps, aiming at producing a solid clone-based assembly with minimal use of whole genome shotgun sequence. The generation of Optical Map assemblies using high molecular weight DNA has informed intra- and inter-chromosomal contig rearrangements in the existing Zv9 assembly, further the data has allowed placement of unlocalised sequence contigs. We have combined these analyses with the generation of new meiotic maps, called GAPMAP based on the MGH panel. GAPMAP was designed to produce small linkage groups to complement the high density SATMAP meiotic map, in low coverage regions. GAPMAP has significantly increased the coverage of meiotic mapping in the assembly and helped immensely with improving the accuracy of the placement of contig-based sequencing data. In addition, approximately 3000 large insert clones have been identified to fill gaps and capture missing genes. These clones have been sequenced using Illumina platforms and assembled via a new pipeline set up to deal with short read data. Sequenced BAC clones are considered appropriate for inclusion into the next assembly release once contigs have been manually ordered and orientated (HTGS_PHASE2). Whole genome shotgun sequence has been required to facilitate clone selection along with subsequent improvement of resultant clone sequence. The GRC are also working to improve the whole genome shotgun sequence with additional data sets sequenced on the PacBio platform, which will further enhance the coverage and structure of individual chromosomes. We will give an overview of the tools, processes and data analyses used for producing the upcoming Zebrafish Reference Genome Assembly, GRCz10. Further information available at www.sanger.ac.uk/resources/zebrafish/genomeproject.html.

488. High-Content Screening Assay for Identification of Chemicals Impacting Cardiovascular Function in Zebrafish Embryos. ***David C. Volz****, Krystle L. Yozzo, Gregory M. Isales, Tara D. Raftery.* Department of Environmental Health Sciences, University of South Carolina, Columbia, SC.
Targeted assays are needed to better evaluate effects of chemicals on organogenesis and begin classification of chemicals by toxicologically relevant modes-of-action. Using transgenic zebrafish (fli1:egfp) that stably express enhanced green fluorescent protein (eGFP) within vascular endothelial cells, we have developed and optimized a 384-well-based high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at sub-lethal, non-teratogenic concentrations. Following static exposure of one embryo per well from 5-72 hours post-fertilization (hpf), automated image acquisition procedures and custom image analysis protocols are used to quantify body length, circulation, heart rate, pericardial area (a biomarker for cardiac looping defects), and intersegmental vessel area within freshly hatched live embryos. After optimizing 72-hpf anesthetization procedures, we evaluated each endpoint across four independent control plates containing 384 initial embryos per plate. Survival and imaging success rates across these plates ranged from 93-99% and 42-74%, respectively. Criteria were then defined for assay success and analysis of treatments, and 10 chemicals were screened for targeted effects on cardiovascular function. Compared to existing zebrafish-based assays, this method provides a comprehensive discovery platform with 1) increased sample sizes; 2) broad concentration-response format; and 3) the ability to identify chemicals that target cardiovascular function at non-teratogenic concentrations.

489. 3D visualization of nerve tracts, blood vessels and fat in juvenile zebrafish using synchrotron microCT. ***X. Xin***[1,2]*, C. Smallwood*[1,2]*, D. Clark*[3]*, X. Xiao*[4]*, P. Riviere*[5]*, K. Cheng*[1,2]. 1) Devision of Experimental Pathology, Penn State College of Medicine, Hershey, PA; 2) Jake Gittlen Cancer Research Foundation, Hershey, PA; 3) Center for In Vivo Microscopy, Duke University Medical Center, Durham, NC; 4) Advanced Photon Source, Argonne National Laboratory, Lemont, IL; 5) Department of Radiology, University of Chicago, Chicago, IL.
The study of 3D tissue architecture and its modification is necessary for a comprehensive understanding of the effects of genes and environment on organisms, but is challenged by limitations of resolution, field of view and tissue opacity. The greatest increases in tissue opacity coincide with organogenesis, making the study of juvenile phenotypes particularly challenging. We describe some of our explorations of the potential of synchrotron microCT to overcome these barriers. To achieve cell resolution and rapid throughput, we have taken advantage of the monochromaticity and high flux of the 2-BM beamline of the Argonne National Laboratory's Advanced Photon Source, a 1 km diameter synchrotron, for microCT studies of juvenile zebrafish. 3D whole fish images of 1.43 micron isotropic voxel resolution were manually generated from segmental scans, totaling about 100GB in size. As shown in animations, we have used different stains and adjustments to volume rendering modes and grey scale histograms to focus visualizations on different organ systems. To illustrate the utility of microCT in the study of organogenesis, we have identified, visualized, and labelled all of the cranial nerves, all the major cranial blood vessels, and the fat cells of individual juvenile zebrafish.

490. Optimized cell transplantation using immune compromised rag2 mutant zebrafish. ***Q. Tang***[1,2]*, N. Abdelfattah*[1,2]*, J. Blackburn*[1,2]*, S. Martinez*[1,2]*, F. Moore*[1,2]*, R. Lobbardi*[1,2]*, M. Ignatius*[1,2]*, J. Moore*[1,2]*, Y. Houvras*[3]*, D. Langenau*[1,2]. 1) Massachusetts General Hospital, Boston, MA; 2) Harvard Stem Cell Institute, Cambridge, MA; 3) Departments of Surgery and Medicine, Weill Cornell Medical College, New York, NY.

Cell transplantation into immune compromised mice has transformed our understanding of cancer. However, transplantation experiments in mice are expensive, utilize small cohorts of animals, and engraftment is difficult to visualize. By contrast, large-scale transplantation of normal and malignant cells into adult zebrafish has now become routine. However, these approaches require that donor cells are from the same syngeneic background as the recipient or that the recipient immune system is transiently ablated to permit engraftment. To date, immune compromised zebrafish have yet to be developed for use as a universal recipient for allograft cell transplantation. Using genome-editing tools for gene inactivation, we designed zinc finger nucleases to target the PHD domain of the zebrafish recombination activating gene 2 (rag2) at similar residues commonly mutated in Omenn's Syndrome, an autosomal recessive severe combined immunodeficiency. rag2 mutant zebrafish exhibited defects in T and B cell receptor rearrangements, resulting in a reduction of functionally mature T and B cells. Homozygous rag2 mutant zebrafish survive into adulthood at correct Mendelian ratios, can be genotyped using the fin-clip method, and robustly engraft a wide range of cell types following transplantation. For example, adult homozygous mutant fish successfully engraft fluorescently labeled kidney marrow and muscle cells from genetically unmatched donors, as well as cancerous tissue including T-cell lymphoblastic leukemia, rhabdomyosarcoma, and melanoma. Finally, we show that fish engrafted with fluorescently labeled T-ALL are responsive to clinically relevant therapies including dexamethasone and gamma-irradiation, suggesting potential utility of the rag2 mutant fish for large-scale in vivo screening of therapeutic agents. In total, the rag2 mutant zebrafish is the first immune compromised zebrafish model that permits robust, long-term engraftment of a wide array of tissues and cancer.

491. *emx1* is Essential for Distal Segment Development in the Zebrafish Pronephros. ***Elvin E. Morales****, Rebecca A. Wingert.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
Vertebrate kidneys are comprised of functional subunits called nephrons. Each nephron can be divided into three parts: the renal corpuscle, which acts as a blood filter; the tubule, which has a series of proximal and distal segments that secrete and reabsorb metabolites; and the collecting duct. The developmental pathways that establish tubule segment identities from renal progenitors remain poorly understood. The zebrafish embryo has a simple two-nephron kidney, the pronephros, which possesses a conserved segment anatomy with other vertebrate nephrons. *emx1* is a homeobox gene that, in cooperation with its paralog *emx2*, is essential for forebrain development, specifically in the telencephalon, and similar expression patterns have been seen in mammals. While *emx1* expression has been reported in the pronephros, its role in nephron development has not been established. Using whole mount *in situ* hybridization, we found that transcripts encoding *emx1* were dynamic in the renal progenitors during nephrogenesis. Interestingly, *emx1* was expressed in a broad domain at 10 somites and became progressively restricted to the distal late (DL) segment by 24hpf. To investigate the function of *emx1*, wild-type zebrafish embryos were microinjected with an *emx1* morpholino at the 1-cell stage, and nephron segment pattern was assessed. *emx1* morphants formed a normal distal domain, marked by *clcnk* expression, but within this domain formed an expanded distal early (DE) segment, marked by *slc12a1*, and a reduced DL segment, marked by *slc12a3*. These data suggest the model that *emx1* is essential to promote the DL segment, and may restrict the DE and/or negiotiate the site of the DE/DL boundary. As our lab recently demonstrated that interplay between retinoic acid (RA) and the transcription factor *mecom* is required for DL formation, future studies will address the relationship between these factors and *emx1*, as well as possible functional redundancy between *emx* factors in nephron patterning. This research can provide valuable new insights into the genetic regulatory networks that direct renal progenitor patterning during nephron formation, and have the potential to reveal conserved mechanisms of kidney development in vertebrates.

492. A Zebrafish Model of Netrin1 Deficiency Reveals Defective Vascular Development Concurrent With Abnormal Endodermal Organ Morphogenesis. ***Robert Opitz***[1]*, Achim Trubiroha*[1]*, Isabelle Vandernoot*[1]*, Marc-Philip Hitz*[2]*, Rasha Abu-Khudir*[2]*, Valérie Désilets*[2]*, Gregor Andelfinger*[2]*, Johnny Deladoëy*[2]*, Sabine Costagliola*[1]. 1) IRIBHM, ULB, Brussels, Belgium; 2) Department of Pediatrics, Centre Hospitalier Universitaire Sainte Justine, Montréal, Québec, Canada.
Mutual interactions between mesoderm and endoderm are vital for cardiovascular development and proper morphogenesis of endoderm-derived organs. Thyroid development represents one exciting model to study this interrelationship given the close coordination of thyroid and cardiovascular development and a high prevalence of thyroid anomalies in mouse and zebrafish models with defective cardiovascular development. Since congenital cardiac defects represent the most frequent extrathyroidal anomalies in patients with congenital thyroid disorders, we set out to screen such patients for rare chromosomal aberrations. In one patient presenting thyroid ectopy and congenital cardiac defects, we detected a *de novo* deletion at the Netrin1 locus. Netrin1 is an axon guidance molecule with additional functions in vascular development and endodermal organ morphogenesis. To examine a potential role of zebrafish netrin1 for thyroid organogenesis, we analyzed developmental expression patterns of *ntn1a* and *ntn1b* in the pharyngeal region and injected zebrafish embryos with *ntn1a* splice-blocking morpholino to study thyroid development in *ntn1a*-deficient embryos. In both, morphant and control embryos, early thyroid development was found to proceed in intimitate contact with the distal ventricular myocardium. However, the tight coordination of subsequent thyroid budding and heart descent, as seen in controls, was disrupted in *ntn1a* morphants which displayed laterally mislocated thyroid primordia. Confocal analyses of *ntn1a* morphants revealed that ectopic localization of thyroid tissue occurred concurrent with specific pharyngeal vasculature anomalies including aortic arch artery defects and abnormal morphogenesis of the hypobranchial artery, a vessel that is important for guiding normal thyroid tissue relocalization. Collectively, our data indicate a novel role of netrin1 function in the regulation of pharyngeal vessel morphogenesis and thyroid organogenesis.

493. Ribosome Biogenesis Factor Bms1-like Is Essential for Liver Development in Zebrafish. ***Yong Wang***[1]*, Yue Luo*[1]*, Yunhan Hong*[2]*, Jinrong Peng*[1]*, Lijan Lo*[1,2]. 1) College of Animal Sciences, Zhejiang University, Hangzhou 310058, China; 2) Department of Biological Sciences, National University of Singapore, Science Drive 4, Singapore 117543, Singapore.
Ribosome biogenesis in the nucleolus requires numerous nucleolar proteins and small non-coding RNAs. Among them is ribosome biogenesis factor Bms1, which is highly conserved from yeast to human. In yeast, Bms1 initiates ribosome biogenesis through recruiting Rcl1 to pre-ribosomes. However, little is known about the biological function of Bms1 in vertebrates. Here we report that Bms1 plays an

essential role in zebrafish liver development. We identified a zebrafish *bms1l*^{sq163} mutant which carries a T to A mutation in the gene *bms1-like (bms1l).*This mutation results in L152 to Q152 substitution in a GTPase motif in Bms1l. Surprisingly, *bms1l*^{sq163} mutation confers hypoplasia specifically in the liver, exocrine pancreas and intestine after 3 days post-fertilization (dpf). Consistent with the *bms1l*^{sq163} mutant phenotypes, whole-mount *in situ* hybridization (WISH) on wild type embryos showed that *bms1l* transcripts are abundant in the entire digestive tract and its accessory organs. Immunostaining for phospho-Histone 3 (P-H3), Proliferating cell nuclear antigen(PCNA) and TUNEL assay revealed that cell cycle arrest rather than cell apoptosis is one of the consequences of *bms1l*^{sq163} giving rise to an underdeveloped liver. Interestingly, the tumor suppressor gene *p53* had a highly expression level in *bms1l*^{sq163} mutant, and the small liver phenotype can be partially rescued in *p53* and *bms1l*^{sq163} double mutant. Therefore, our findings demonstrate that Bms1l is necessary for zebrafish liver development.

494. Analysis of Nephron Composition and Function in the Adult Zebrafish Kidney. *Kristen K. McCampbell, Kristin Springer,* ***Rebecca A. Wingert****.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.
The zebrafish kidney is an excellent system for renal regeneration and disease studies, as it is composed of functional units known as nephrons that contain highly conserved proximal and distal tubule segments similar to other vertebrates including mammals. After zebrafish nephrons incur damage, there is robust epithelial regeneration within existing nephrons and new nephrons are produced from renal progenitors in a process known as neonephrogenesis. To date, the mechanisms responsible for these kidney regeneration phenomena remain poorly understood and a major limitation in the field has been the paucity of methods to label adult nephron cell populations. Here, we present a novel set of labeling methods that can be used in whole mount preparations or tissue sections to gage renal composition and assess nephron functionality. We show that alkaline phosphatase (AP) is a pan-proximal marker while fluorescent dextran uptake labels the proximal convoluted tubule. In contrast, the rhodamine-tagged lectin *Dolichos biflorus* agglutinin (DBA) marks the distal tubule. As DBA is mutually exclusive to AP staining, these labels provide a way to distinguish pan-proximal versus pan-distal stretches in the nephron. Throughout the implementation of these fluorescent stains using novel whole mount procedures and cryostat histological techniques, we validated the labels by extensive comparisons to the expression domains of solute transporter genes that uniquely identify each nephron segment. These analyses have provided new insights into nephron anatomy in the adult zebrafish kidney. This information is widely applicable to the future phenotypic characterization of adult zebrafish kidney injury paradigms, which include but are not limited to, nephrotoxicant exposure regimes or genetic methods of targeted cell death such as the nitroreductase mediated cell ablation technique. Further, these methods could be used to study genetic perturbations in adult kidney formation and could also be applied to assess renal status during chronic disease modeling.

495. Do the Valve Endocardial Progenitors Originate from a single Zebrafish Blastula HPRG1+ Cell? ***Xiushan Wu***. The Center for Heart Development, Hunan Normal University, Changsha, Hunan, China.
The arguments regarding the origin of the endocardial progenitors remain unresolved (Harris and Black, 2010). Here, we have identified a gene, tentatively named HPRG1 (heart progenitor regulation gene 1), through a large-scale screen of Drosophila mutants. The gene is expressed in heart valves in zebrafish and its expression pattern is conserved in mice. Knockdown of the gene resulted in a valve defect, suggesting it is involved in endocardial valve development. It is known that Isl1 or GATA4 positive cells are capable of differentiating into two cell types, endocardial and cardial progenitors, and NKx2.5 is the direct activator of endocardial master regulator Etv2. Our results indicated that HPRG1 is expressed in a novel type of mesodermal progenitor cells that are co-expressed with each master regulators and HPRG1 activates the expressions of GATA4 and NKx2.5 and inhibits the expression of Isl1. It is specially interesting that HPRG1 determines the fate of a single cell of the 128-cells at Zebrafish blastula stage, suggesting that it is a fate-determining gene. Thus, the HPRG1 positive blastula cells provide an appropriate experimental system for exploring the specification mechanism of the endocardial progenitors. A mechanism for heart valve progenitor specification beginning with HPRG1 through GATA4, Isl1 and NKx2.5 is under investigation. Key words: zebrafish, blastula, HPRG1 pre-progenitors, cardiac mesoderm, endocardial valve fate.

496. The roles of zebrafish *mesp* genes in somitogenesis. ***Taijiro Yabe***[1]*, Chimwar Wanglar*[1]*, Kazuyuki Hoshijima*[2]*, Takashi Yamamoto*[3]*, Shinji Takada*[1]. 1) National Institute for Basic Biology, Okazaki, Japan; 2) University of Utah, Salt Lake City, UT, USA; 3) Hiroshima University, Hiroshima, Japan.
Somites, the epithelial block of mesodermal cells, are metameric structure transiently formed adjacent to notochord and sequentially budded off from the most anterior end of presomatic mesoderm (PSM) during vertebrate development. The somite formation is considered to be regulated by the “clock and wave front model”, in which the periodical generation of somite is mediated by the integration of temporal information and positional information provided by the cyclic activation Notch signaling and posterior movement of anterior edge of Fgf activity respectively. Previous studies revealed mouse *Mesp2* has essential roles for multiple processes of somitogenesis including determination of position of segment boundaries, generation of somite boundary structure and establishment of rostro-caudal polarity in each somite. Although 4 *mesp* genes have been identified in zebrafish genome, their roles in somitogenesis still remained unclear. To address this problem we generated mutant fish carrying the frame shift mutation in all of *mesp* genes using TALEN mediated mutagenesis and analyzed its phenotype. *mesps* quadruple embryo exhibited the caudlized somite similar to mouse *Mesp2* mutant. These mutant embryos also showed disrupted superficial horizontal myoseptum formation probably resulting in the mis-migration of pigment cells and lateral line primordia in later development. Also we would like to discuss the roles of *mesp* genes in zebrafish somite boundary formation.

497. Genetic and cellular mechanisms of hepatic cystogenesis in a novel zebrafish model of polycystic liver disease. ***Chunyue Yin***[1,2], *Kimberley Evason*[3], *Jillian Ellis*[1], *Allison Ross*[1], *Ian Fiddes*[2], *Shiva Kumar Shanmukhappa*[4], *Kevin Bove*[4], *Nikolay Ninov*[2], *Jiandong Liu*[2], *Didier Stainier*[2,5]. 1) Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital, Cincinnati, OH; 2) Department of Biochemistry and Biophysics, UCSF, San Francisco, California; 3) Department of Pathology, UCSF, San Francisco, California; 4) Department of Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; 5) Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.
Polycystic liver diseases (PCLDs) are genetic disorders of the biliary epithelium, characterized by progressive formation of fluid-filled cysts throughout the liver. Hepatic cysts result in massive enlargement of the liver. Progression to secondary fibrosis, cirrhosis, or cholangiocarcinoma also causes significant morbidity and mortality. However, the cellular and molecular causes of hepatic cystogenesis are poorly understood. In a forward genetic screen, we identified a zebrafish mutant that develops bile-filled hepatic cysts during larval stages. Phenotypic analyses revealed that the mutant cholangiocytes exhibit a modest increase in proliferation, similar to what has been reported in studies of rodent PCLD models. As shown by transmission electron microscopy, the rough endoplasmic reticulum in the mutant cholangiocytes is severely dilated. Mutations in genes involved in ciliogenesis have been shown to cause human PCLDs. We examined the localization of cilia markers and found that the primary cilia are much shorter in the mutant cholangiocytes compared to wild-type. Meanwhile, the mutant cholangiocytes contain a clearly expanded microtubule network compared to wild-type. Lastly, through time-lapse live imaging experiments, we showed that whereas the wild-type cholangiocytes extend cellular protrusions that fuse to form bile ducts, such behaviors failed to occur in the mutants. In summary, we have established a novel animal model of PCLDs and revealed the involvement of primary cilia and microtubules in cholangiocyte behavior and cystogenesis. Currently, we are dissecting the nature of the underlying mutation to better understand the molecular regulation of PCLDs.

498. Inhibition of VEGF Signaling Ameliorates Hepatic Steatosis in Acute Alcoholic Liver Injury in Zebrafish. ***Changwen Zhang***, *Chunyue Yin.* Division of Gastroenterology, Hepatology and Nutrition , Cincinnati Children's Hospital Medical Center, Cincinnati, OH. 45220.
Background and Aims: Alcoholic liver disease(ALD) is among the most common causes of chronic liver disease worldwide. One of the main responses to the ethanol-induced liver injury is the activation of hepatic stellate cells (HSCs), whereby these quiescent cells differentiate into proliferating myofibroblast-like cells and produce excessive extracellular matrix to replace the normal hepatic tissue. These activated HSCs(aHSCs hereafter) are the major fibrogenic cell type in the liver. We developed a transgenic zebrafish line *Tg(Hand2:EGFP)* which express GFP in HSCs, and we utilized an acute alcoholic liver injury model to study the biology of HSC in the pathophysiology liver. It has been shown that the expression of vascular endothelial growth factor (VEGF) and VEGF receptors are increased in the mouse liver steatosis and fibrosis model. So we hypothesize that VEGF might contribute to the activation of HSCs and fibrogenesis. Methods: Zebrafish larvae were treated with 2% EtOH from 96 and 120hpf, and then transferred to egg water with either DMSO as control, or inhibitors of VEGFR. Liver tissues of the zebrafish were analyzed by immunohistochemistry. Results: Inhibition of VEGF signalling attenuated the increase of aHSC number after acute ethanol treatment and ameliorated hepatic steatosis. Conclusion: Our preliminary data suggest that VEGF may be an important contributor to alcohol-induced liver steatosis by regulating the increase of aHSC number.

499. Evolution of post-embryonic neural crest lineage contribution to adult pigment pattern in *Danio* fishes. ***Jessica E. Spiewak***, *Dave M. Parichy.* Biology, University of Washington, Seattle, WA.
The diversity of vertebrate form is in large part attributable to changes in the patterning of neural crest cells and their derivatives. Pigment patterns of fishes in the genus *Danio* are a tractable system in which to uncover how cellular and molecular changes in neural crest lineages lead to phenotypic diversity among closely related species. Adult zebrafish, *D. rerio*, have horizontal stripes of black melanophores derived mainly from post-embryonic, neural-crest derived latent precursors. However, the closely related species *D. nigrofasciatus* has an evolutionary reduction in latent-precursor derived melanophores, and its pigment pattern is composed largely of persisting embryonic melanophores. Here, we identify evolutionary changes in endothelin signaling as a candidate mechanism for altering the relative contributions of embryonic and post-embryonic neural crest lineages. A previous study indicated that *endothelin receptor b1* (*ednrb1*) mutant zebrafish have a pigment pattern similar to *D. nigrofasciatus*, whereas cell transplants showed the differences between species to be non-autonomous to melanophore lineages. We therefore assessed expression of genes encoding Ednrb1 ligands and found much lower abundance of *endothelin-3b* (*edn3b*) transcript in *D. nigrofasciatus* than in *D. rerio*. By analyzing gene expression in *D. rerio* x *D. nigrofasciatus* hybrids, we further showed that evolutionary changes in *edn3b* expression have resulted from *cis*-regulatory evolution at this locus. We are now testing whether *D. rerio edn3* can restore latent-precursor derived melanophores to *D. nigrofasciatus*, as well as the roles of endothelin signaling—and its abrogation—in the development of melanophores and other lineages in these species.

500. Mechanisms of Nutrient Availability in the Adaptive Plasticity of Anamniote Hatching. ***Rebecca Thomason***[1], *Dhivya Kumar*[2], *Jason Sloan*[4], *Richard Mains*[3], *Betty Eipper*[2], *Jonathan Gitlin*[1]. 1) Marine Biological Laboratory, Woods Hole, MA; 2) Molecular Biology and Biophysics Department, University of Connecticut Health Center, Farmington, CT; 3) Department of Neuroscience, University of Connecticut Health Center, Farmington, CT; 4) Department of Biology, Hunter College, New York City, NY.
The timing of hatching in anamniotes is a critical developmental event that is adaptively plastic in response to numerous environmental conditions. To elucidate the role of nutrient availability in this process, we took advantage of the well-defined phenotypes in zebrafish with genetic and nutritional differences in the metabolism of copper, a trace element essential for development. Treatment of developing embryos with neocuproine, a membrane permeable copper chelator, resulted in a dose-dependent decrease in the rate and percent of hatching at 96 hours post fertilization. Furthermore, the effect of neocuproine on the rate and percent of hatching was increased in copper transport deficient mutant embryos when compared to wild type siblings. These data suggest that a specific copper-dependent enzymatic

pathway is involved in the hatching process. Peptidylglycine a-amidating monooxygenase is an evolutionarily conserved cuproenzyme essential for the biosynthesis of several peptides required in neuroendocrine physiology. Consistent with a direct role for this cuproenzyme in the copper-dependent effect on hatching, antisense abrogation of peptidylglycine a-amidating monooxygenase activity resulted in a similar reduction in the rate and percent of hatching at 96 hours post fertilization. Importantly, this effect was further increased by a dose of neocuproine that alone was without impact on hatching. Peptidylglycine a-amidating monooxygenase activity assays reveal a marked decrease in enzymatic activity in morpholino-injected embryos when compared to uninjected and control embryos. Remarkably, the development and morphology of the hatching gland is normal, suggesting a role for peptidylglycine a-amidating monooxygenase in other processes involved with hatching. Taken together, these studies reveal a critical role for copper availability in the adaptive plasticity of hatching and define a unique neuroendocrine mechanism mediating this process.

501. Evolution and functional divergence of zebrafish cryptochrome genes. *Chao Liu, Jia Hu, Chunxiang Qu, **Han Wang***. Center for Circadian Clocks, Soochow University, Suzhou, Jiangsu, China.
Cryptochrome (*Cry*) genes play important roles in circadian regulation and photoreception. While zebrafish are known to have six *cry* genes, their evolutionary relationships and the mechanisms underlying their functional divergence are not yet fully resolved. Here we aim to elucidate the mechanisms underlying zebrafish *cry* evolution and functional divergence. Using phylogenetic and syntenic analyses, we found that following the two-round vertebrate genome duplication (VGD) and subsequent gene loss, teleost fish retained three subgroups of *cry* genes including *cry1*, *cry2* and *cry3*; before the third-round teleost genome duplication (TGD), local duplication of *cry1* likely occurred in teleost fish, which generated *cry1.1* and *cry1.2*; and following TGD and subsequent gene loss, zebrafish retained *cry1.1a*, *cry1.1b*, *cry1.2a*, *cry1.2b*, *cry2* and *cry3*. *In situ* hybridization and quantitative real-time PCR showed that these six *cry* genes are rhythmically expressed with distinct patterns in a robust manner, suggesting that these cry genes have evolved divergent functions. *In vitro* cell transfection assays showed zebrafish possess two types of Cry proteins, inhibitory Cry (IC) including Cry1.1a, Cry1.1b, Cry1.2a, and Cry1.2b that are potent repressors of Clock:Bmal heterodimers-mediated transcription, and non-inhibitory Cry (NIC) including Cry2 nor Cry3 that cannot repress Clock:Bmal heterodimers-mediated transcription. We also found possible mechanisms underlying non-inhibition of Cry2 and Cry3, i.e., Cry3 is a cytoplasmic protein, and Cry2 cannot effectively bind to the Bmal1 protein, even though it can enter inside the nucleus. In addition, Cry1.1a and Cry1.2b appear to evolve different nuclear localization signal (NLS) sequences. Taken together, these findings contribute to the growing body of knowledge of Cry evolution, and set the stage for studying their functions. *The first three authors contributed equally to this work.

502. A zebrafish insertional screen identifies genes required for T cell development. ***Christoph Seiler**[1], Yong Zang[1], Susan Shinton[1], Jorune Balciuniene[2], Gaurav K. Varshney[3], Matthew Lafave[3], Shawn M. Burgess[3], Darius Balciunas[2], Dietmar Kappes[1], Richard Hardy[1], David Wiest[1], Jennifer Rhodes[1]*. 1) Fox Chase Cancer Center, Philadelphia, PA; 2) Temple University, Department of Biology , Philadelphia, PA; 3) National Human Genome Research Institute, NIH, Bethesda, MD.
Most of our knowledge of immune cell development has been acquired by studying mouse models or mammalian immune cells in culture. The innate and adaptive immune systems of the zebrafish are highly conserved compared to mammals, suggesting that discoveries of genes important for immune cell development or function in zebrafish will be relevant to humans. To find new genes important to this process, we took advantage of a recently developed transposon based insertional mutagenesis screen. In this approach “gene-breaking” vectors label cells expressing the targeted gene with green fluorescent protein (GFP). This strategy permits immediate evaluation of the expression pattern of the affected gene; carriers of gene-breaking cassettes can be identified accordingly and incrossed to generate homozygous mutants. Here, we describe the identification of 16 lines that have expression in embryonic immune cells at day 2 or day 6. Flow cytometric sorting of 6-week-old fish indicates that the fluorescently tagged cells are present in circulation in juvenile larvae in 13 of these lines. Gene expression analysis of FACS sorted GFP-positive cells was used to further define their immune cell identity. We used high-throughput sequencing in collaboration with the NIH, inverse PCR and RACE strategies to identify the disrupted genes in most of the lines. Importantly, incross-analysis of 3 lines revealed a defect in T cell development and could be phenocopied by morpholino analysis. The disrupted genes have not been previously identified as important regulators of lymphopoiesis, suggesting that we identified novel factors required for thymus cell development.

503. Role of the sclerotomal genes shisa2 and pdgfrl in hematopoietic stem cell specification. ***Sarah Shore**, Wilson Clements.* Department of Hematology, St. Jude Children's Research Hospital.
Hematopoietic stem cells (HSCs) are self-renewing progenitor cells that produce all blood and immune cells during life, and are the clinically relevant component of bone marrow transplants. A better understanding of how HSCs are specified during development might inform attempts to produce HSCs in vitro, a key medical goal that is not currently possible. HSCs first arise from endothelial cells in the dorsal aorta of vertebrates. Our previous research showed that a secreted signaling molecule, Wnt16, is required for HSC specification. Wnt16 does not act directly on HSC precursors, but rather through a complex series of poorly understood relay signals. Wnt16 knockdown animals also have defects in the sclerotome compartment of the somite—which produces vascular smooth muscle cells that surround the dorsal aorta—suggesting that sclerotome genes might direct formation of the HSC specification niche or act as specification signals. We cloned two candidate sclerotome genes, shisa2, which encodes a putative antagonist of Wnt and Fgf signaling, and pdgfrl, which encodes a secreted homologue of the growth factor receptor Pdgfr, and confirmed expression by whole mount in situ hybridization. In gain-of-function analyses, shisa2 over-expression produced no phenotype, but pdgfrl overexpression yielded embyros with decreased head size and tail malformations, suggesting an ability to interact with signaling pathways that regulate head specification and convergence/extension, including the Wnt pathway. Interestingly a previous protein-interaction screen indicated that Pdgfrl physically interacts with the atypical Wnt receptor Musk. In the future, we will biochemically confirm Pdgfrl/Musk interaction during embryonic development, and examine the

effects of pdgfrl knockdown on somite patterning and HSC specification. These studies will help to define the molecular factors that pattern the HSC specification niche.

504. Zebrafish embryonic stromal trunk (ZEST) cells support multilineage hematopoiesis. ***David L. Stachura***, *Clyde A. Campbell, David Traver.* Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA.
Mature hematopoietic cells are constantly generated by the renewal and subsequent developmental restriction of hematopoietic stem cells (HSCs), which differentiate into lineage-restricted progenitors responsible for generating the full repertoire of blood and immune cell types throughout an organism's lifetime. While forward genetic screens in zebrafish have been utilized to identify genes essential for the generation of primitive blood and the emergence of HSCs, they have not uncovered genes essential for hematopoietic stem and progenitor cell (HSPC) proliferation and differentiation, primarily due to a lack of methodologies to functionally assess these processes. We previously described techniques to test the developmental potential of HSPCs by culturing them on zebrafish kidney stromal (ZKS) cells, derived from the main site of hematopoiesis in adult teleosts. Here we describe a primary stromal cell line we named zebrafish embryonic stromal trunk (ZEST) cells, derived from tissue surrounding the embryonic dorsal aorta, the site of HSC emergence in the developing fish. ZEST cells encouraged HSPC differentiation down the myeloid, lymphoid, and erythroid pathways when assessed by morphological and qRT-PCR analyses. Additionally, ZEST cells significantly expanded the number of cultured HSPCs in vitro, indicating that these stromal cells are supportive of both HSPC proliferation and multilineage differentiation. Examination and comparison of the transcriptome of ZKS and ZEST cells indicate that these two different hematopoietic niches upregulate many signaling pathways likely essential for HSPC proliferation and differentiation. Further characterization of ZEST cells should prove to be invaluable in understanding the complex signaling cascades instigated by the embryonic hematopoietic niche required to expand and differentiate HSPCs. Elucidating these processes and identifying possibilities for the modulation of these molecular pathways should allow the in vitro expansion of HSPCs for a multitude of therapeutic uses.

505. Identification of *Hox* Genes Controlling Thrombopoiesis in Zebrafish. ***H. Sundaramoorthi***, *P. Jagadeeswaran.* Biological Sciences, University of North Texas, Denton, TX.
Thrombocytes are nucleated blood cells functionally equivalent to mammalian platelets which play a central role in hemostasis. They also possess megakaryocyte features because thrombocytes and megakaryocytes have similar transcriptional machinery. *Hox* genes have already been shown to play a role in megakaryocyte development. However, a comprehensive genome-wide scan of all *hox* genes that play a role in megakaryopoiesis has not been done. Since thrombocytes represent megakaryocytes, the goal of this study was to comprehensively knockdown *hox* genes to identify specific *hox* genes involved in in zebrafish thrombopoiesis. Therefore, we initially selected five *hox* genes, *hoxa10b*, *hoxb2a*, *hoxc5a*, *hoxc11b*, and *hoxd3a* from our earlier microarray analysis. The selected genes showed a level of mRNA expression in thrombocytes that was more than two times greater than that found in erythrocytes. We tested for the differential expression of these *hox* genes in both young and mature thrombocytes by RT-PCR. We found that *hoxc11b* was expressed only in young thrombocytes and that the remaining four genes were expressed in both young and mature thrombocytes. The techniques available to perform knockdowns of these genes and test for the number of thrombocytes were either cumbersome or required the breeding and production of fish in which thrombocytes are GFP labeled. Therefore, we used the white cell fraction of wild-type fish blood fluorescently labeled with mepacrine to establish a flow cytometry method of counting thrombocytes. We injected adult fish with a control morpholino piggybacked with a standard oligonucleotide antisense to the central portion of the mRNA for each of these *hox* genes. We counted thrombocytes at 48 hours post injection and found that knockdown of *hoxa10b, hoxb2a, hoxc5a*, and *hoxd3a* showed reduction in the thrombocyte counts while knockdown of *hoxc11b* increased thrombocyte counts. Using this flow sorting method we then screened another 47 *hox* genes in the zebrafish genome and found that knockdown of *hoxa9a* and *hoxb1a* also resulted in decreased thrombocyte number. In conclusion we found six *hox* genes that are positive regulators and one *hox* gene which is a negative regulator for thrombocyte development.

506. The gene-trap line *qmc551* suggests an important role for the Gfi1 transcription factor during haematopoietic stem cell formation in the vertebrate embryo. ***Deniz Ucanok***[1], *Roshana Thambyrajah*[1], *Maryam Jalali*[1], *Chris Moore*[1], *Robert Wilkinson*[2], *Martin Gering*[1]. 1) The University of Nottingham, Queen's Medical Centre, Nottingham, UK; 2) The University of Sheffield, Medical School, Beech Hill Road,Sheffield, UK.
In vertebrates, haematopoietic stem cells (HSCs) form during embryogenesis and maintain our blood system throughout life. Using a transposon-based gene trap approach in zebrafish, we identified a transgenic line called *qmc551* that expresses an egfp reporter gene at the earliest stages of HSC development, the time when HSCs form from haemogenic endothelial cells in the ventral wall of the dorsal aorta (vDA). These eGFP-positive cells co-express the haematopoietic transcription factors c-Myb and Runx1 and undergo endothelial to haematopoietic transition (EHT). While most of the haemogenic endothelial cells leave the endothelium to enter the mesenchyme underneath the dorsal aorta and join blood circulation via the underlying vein, we show for the first time that individual vDA cells bud into the lumen of the dorsal aorta to join the circulation, a process that more closely resembles HSC formation in the mammalian embryo. Once in circulation, the eGFP-positive cells seed the caudal haematopoietic tissue before they migrate to the larval thymus and kidney. In the adult *qmc551* transgenic, the kidney retains eGFP expression in immature blood progenitors, granulocytes and lymphoid cells. The eGFP expression pattern in *qmc551* transgenics is mediated by a gene-trap transposon located in the *gfi1aa* gene. In fact, eGFP expression in our transgenics nicely recapitulates endogenous *gfi1aa* expression in the haematopoietic system as well as in other organs. Although our data hint at an important role for Gfi1aa in EHT and adult haematopoiesis, homozygous transgenics are viable and fertile, suggesting that Gfi1aa expression is either not affected or that its loss is compensated for in the transgenic fish. In this presentation, we will provide detailed information on the regulation of *gfi1aa* expression in wild-type and *qmc551* transgenic embryos and give a progress report on experiments aimed to elucidate the role of Gfi1 in HSC formation during vertebrate embryogenesis.

507. The Dynamic Process of Microglial Colonization of the Developing Zebrafish Brain. *Jin Xu, Tienan Wang,* ***Zilong Wen****.* The Hong Kong University of Science and Technology, Kowloon, Hong Kong.
Microglia are the resident macrophages of the central nervous system (CNS), and they play important roles in the regulation of neuron development and neuron function. Yet, the routes and the dynamic behaviors of microglial colonization of the CNS remain largely undefined. Here, by utilizing in vivo time-lapse imaging and genetic amenable ability of zebrafish, we show that microglia precursors enter and colonize the optic tecum, where the majority of microglia reside during early zebrafish development, via the bi-lateral periphery of the brain or passing through the ventral brain. The colonization process of the optic tectum by microglial precursors is dynamic and involves two distinctive steps, homing and settling, both of which are driven by the neuronal cell death in the brain and independent of the circulation. Our work provides the cellular basis for the in vivo observation and mechanistic study of microglial colonization of the brain.

508. Gcsf-Chr19 promotes neutrophils migration to damaged tissue through blood vessels in zebrafish. ***Constanza Zuñiga-Traslaviña****, JA Galdames, K Bravo-Tello, CG Feijóo.* Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello, Santiago, Chile.
Granulocyte colony-stimulating factor (Gcsf) is an essential cytokine that regulates proliferation and differentiation of granulocytes and macrophages from hematopoietic stem and progenitor cells. In mammals Gcsf has been also identified as a key factor inducing neutrophils release from the bone marrow into the blood circulation. In zebrafish, the genome sequence analysis indicates that there are two gcsf genes, gcsf-chr12 and gcsf-chr19, located chromosome 12 and 19 respectively. It has been reported that Gcsf-Chr12 participates in myelopoiesis but is not necessary for neutrophils migration during an inflammation process. On the other hand, not much is known about the function of Gcsf-chr19, recently it has been demonstrated that this cytokine has the ability to induce granulocytes proliferation. In this research, we analyzed if different type of mechanical damage triggered neutrophils migration throug blood vessels and if Gcsf-Chr19 is implicated in this process. Our results indicated that after an intense damage, Gcsf-Chr19 transcript levels increase and it knock down decrease the number of neutrophils that arrives through the blood vessels to the wound, indicating that it is participating in the process.

509. Non-hatching decapsulated *Artemia* cysts as a replacement to *Artemia* nauplii in zebrafish culture. ***Marc T. Tye****, Dana Rider, Elizabeth A. Duffy, Adam Seubert, Brogen Lothert, Lisa A. Schimmenti.* University of Minnesota Zebrafish Core Facility, Department of Pediatrics, University of Minnesota-Twin Cities, Minneapolis, MN.
Feeding *Artemia* nauplii to zebrafish is a common practice for most research facilities. Culturing live feed can be time consuming and requires additional equipment to be purchased, maintained and cleaned. Non-hatching decapsulated *Artemia* cysts (decaps) are a commercially available product that can be fed directly to fish. Several other ornamental fish species have been successfully cultured using decaps. Replacing *Artemia* nauplii with decaps could reduce the overall time and costs associated with the operation of a zebrafish facility. The objective of this study was to determine if decaps could be a suitable replacement to *Artemia* nauplii in zebrafish culture. Segrest wildtype zebrafish (28 days post fertilization) were fed one of three treatments: decaps, nauplii and standard (nauplii+pellet food) for approximately 18 weeks. Survival, growth (length and weight) and embryo production were analyzed between the treatments. Fish receiving the decap diet showed significantly higher growth and embryo production when compared to the fish receiving the nauplii diet. Fish receiving the standard treatment were found to perform similarly to fish receiving the decap treatment. It was determined that non-hatching decapsulated *Artemia* cysts can be used as a replacement to *Artemia* nauplii in zebrafish culture.

510. SDF1a/CXCR4b axis mediates neutrophil retention during development and inflammation. *Oscar Peña,* ***Margarita Parada****, Salomé Muñoz, Susana Paredes, Carlos Rubilar, Miguel Allende.* FONDAP Center for Genome Regulation, University of Chile, Santiago.
Stromal derived-cell factor 1 (SDF1, CXCL12) mediated activation of the chemokine receptor CXCR4 is involved in developmental and pathologic processes, including primordial germ cell migration, invasive migration of cancer cells and leukocyte trafficking. Recent evidence suggests a role for SDF1 in neutrophil retention in bone marrow, as CXCR4 mutations are associated with neutropenia. Here, we provide evidence for a role of SDF1/CXCR4 signaling in neutrophil motility during inflammation and resolution. We used mutant zebrafish larvae carrying truncated versions of SDF1a (*medusa*) and CXCR4b (*odysseus*). Neutrophils were visualized with Sudan Black staining or by using transgenic zebrafish whose neutrophils express GFP (*TgBAC(mpx:GFP)*). Inflammation was induced by tail trasection or by injecting bacteria in the otic vesicle of larvae. In vivo time extended microscopy was carried out in larvae subjected to tail transection, and neutrophil dynamics during inflammation was studied. At early larval stages sudan black staining shows no difference in neutrophil distribution between mutant larvae and their siblings in both *medusa* and *odysseus* mutants. However, 7 dpf mutant larvae show significantly less granulocytes in the pronephros. Quantification of circulating neutrophils in transgenic mutant larvae show a progressive increase in medusa larvae compared with their siblings. Quantification of neutrophils recruited 4 hours after tail transection shows a significant increase of infiltrating neutrophils in mutant larvae compared with their siblings. Neutrophil clearance during inflammation resolution is also regulated by SDF1a, as SDF1a mutants show a significant increase in recruited neutrophils 24 hours after tail transection. We provide evidence for a role of SDF1a/CXCR4b signaling in neutrophil release to recruitment during inflammation and its resolution, respectively. Our results support SDF1a as a retention signal for neutrophils in the caudal hematopoietic tissue, suggesting that SDF1a restricts the number of responding neutrophils upon tissue injury. Grant sponsors: FONDAP 15090007.

511. Soybean meal-based diet induces intestinal inflammation in zebrafish. ***Pilar E. Ulloa***[1]*, Adrián Hernández*[2]*, Patricio Dantagnan*[2]*, Carmen G. Feijoo*[1]. 1) Departamento de Ciencias Biológicas, Universidad Andrés Bello, Chile; 2) Núcleo de Investigación en Producción Alimentaria, Universidad Católica de Temuco, Chile.
Dietary impacts on health is one of the oldest concepts in medicine; however our understanding on how individual dietary components can affect specific illnesses is far from being elucidated. Inflammatory Bowel Diseases (IBD) is a broad term that describes conditions with chronic or recurring inflammation of the gastrointestinal tract. The two most common IBD are ulcerative colitis and Crohn's disease.

Traditionally, mammalian models have been chosen to develop research in IBD, generating major advances in the knowledge on this pathology. Otherwise, in the last years the zebrafish has become a popular model to study innate immune response and inflammation, including diseases such as IBD. Specifically, to study IBD a model in which enteritis is induced by a chemical is used. In order to address the problematic from a different point of view, we developed a singular approach, where the intestinal inflammation is triggered by a diet that contains soybean meal. This legume is composed by soy protein and anti-nutritional factors, which exerts an inflammatory action in the intestine of different animals, including zebrafish. Using this strategy, and incorporating different compound to the diet, we expect to identify additives that exert "intestinal protective activity" over food similar to soybean. To perform our research, we took advantage of the Tg(Bacmpx:GFP) transgenic line, where neutrophils are fluorescently labeled, allowing us to make in vivo analysis. We analyzed different additives and found that diet supplemented with yeast b-glucan showed a significant decrease in the number of neutrophils localized in the intestine, suggesting an intestinal protective effect. Transcriptional levels of pro-inflammatory genes as well as genes involved in gut mucosal barrier defense do not showed conclusive results. Due to identifying the correct timing in which to perform the qPCR is difficult and laborious and to complement our in vivo analysis, we are, developing assays in order to evaluate intestine lipid absorption capacity as well as intestinal epithelium integrity. Fondecyt N°3130664.

512. Modelling MODY in Zebrafish. ***Rachel A Watson***, *Inês Barroso*. Human Genetics, Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, United Kingdom.
Maturity Onset Diabetes of the Young (MODY) is an autosomal dominant form of diabetes that normally develops before 25 years of age, caused by primary insulin secretion defects. There are currently 11 genes identified which, when mutated, can cause MODY. The most commonly mutated are HNF1a, HNF1b, HNF4a and GCK. We have utilised morpholino (MO) knockdown of the orthologs of these four genes (of which there are two for HNF1b) in an attempt to investigate the effect of MO gene knockdown as a model of MODY in zebrafish. We aimed to assess any alterations in endocrine pancreas function, and evaluate the utility of these types of MO knockdown studies for future studies of novel candidate genes from human genetic discoveries. Insulin expression was assayed using RT-qPCR and through injection of the MOs into the ins:mCHERRY transgenic zebrafish line. These studies revealed a large variation between clutches in insulin expression during the first five days post-fertilisation, with the effect of MOs targeting the MODY genes also differing. These results provide a valuable insight into the limitations of MO use, suggesting that any future modelling of this disease in zebrafish be designed using a permanent genome editing tool to allow investigation of the phenotype in older zebrafish.

513. Biological function of selenium containing imidazole compound, selenoneine, in selenium redox metabolism. ***M. Yamashita***[1], *S. Imamura*[1], *T. Yabu*[2], *K. Ishihara*[1], *Y. Yamashita*[1]. 1) Food Safety Assessment Group, National Research Institute of Fisheries Science, Yokohama, Kanagawa, Japan; 2) Nihon University, Fujisawa, Japan.
The novel Se-containing strong antioxidant selenoneine, 2-selenyl-N_a,N_a,N_a-trimethyl-L-histidine, has recently been discovered to be the predominant form of organic Se in tuna blood (Yamashita & Yamashita, JBC, 285, 18134, 2010). A substantial proportion of the total amount of selenium is present as selenoneine in the muscles of ocean fish. This compound is thought to play a key role in the Se redox antioxidant mechanism in animal cells. Cell growth of human cultured cells were enhanced in the presence of selenoneine at 5-100 nM, and GPx1 gene expression was induced in dose-dependent manner. The uptake of selenoneine was mediated by organic cations/carnitine transporter-1 (OCTN1, SLC22A4). Selenoneine in culture medium was incorporated into human cultured cells and zebrafish embryo by OCTN1. When zebrafish embryos were cultured in the presence of 0.1 mM [^{76}Se]-selenite Na in Hank's medium at 28.5°C for 3 days, ^{76}Se-labeled selenoneine was detected in the methanol extract of the embryos by HPLC-ICP-MS analysis. Although methylmercury (MeHg) accumulation and toxicity were reduced in the presence of selenite or selenoneine, such MeHg detoxifying function was blocked in the OCTN1-knockdown embryos. Therefore, selenoneine was found to be biologically synthesized in the zebrafish embryos in vivo.

514. The biological role of heme transporters in zebrafish erythropoiesis. ***Jianbing Zhang***, *Iqbal Hamza.* Department of Animal and Avian Sciences, Univeristy of Maryland, College Park, College Park, MD.
Heme is an essential cofactor for proteins involved in various biological functions. The biosynthesis and utilization of heme has been well-characterized, however, the biological pathways of inter- and intra-cellular heme transport remain poorly understood. We previously identified HRG-1 (Heme Responsive Gene-1) and MRP-5 (Multidrug Resistance Protein-5 or ABCC-5) as heme transporters in the roundworm C. elegans. To determine their functions in a vertebrate model system, we exploited zebrafish which contain two homologs of hrg1. As observed for the worm homologs, expression of hrg1a and hrg1b in a heme synthesis deficient S. cerevisiae strain (hem1D) rescues yeast growth, whereas mrp5 reduces yeast growth in the presence of exogenous heme. Whole-mount in situ hybridization (WISH) shows zebrafish hrg1 and mrp5 mRNA are expressed throughout the embryo, including central nervous system. Knockdown of hrg1 by antisense morpholino results in severe anemia with depletion of mature red blood cells, together with hydrocephalus and shorten yolk extension. However, hrg1b knockdown only causes mild anemia. Surprisingly, knockdown of mrp5 by either translation- or splice-blocking morpholinos also results in anemic phenotype. WISH staining show that expression of gata1 is significantly decreased in erythroid progenitor cells, suggesting that mrp5 knockdown affects the specification of erythroid lineage during hematopoietic development. To further delineate the roles of hrg1 and mrp5 in heme transport, we are generating transcriptional and translational reporter constructs using BAC recombineering. We are also employing TALEN and CRISPR/Cas9 genome editing technologies to generate hrg1 and mrp5 null mutants to determine the role of single and double mutant fish in vertebrate erythropoiesis.

515. Migration of Muscle progenitors into the Zebrafish Pectoral Fin. ***Wouter H. B. Masselink***[1], *Thomas E. Hall*[1,3], *Jin C. Wong*[2], *Boyin Liu*[2], *Jing Fu*[2], *Peter D. Currie*[1]. 1) Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia; 2) Department of Mechanical and Aerospace Engineering, Faculty of Engineering, Monash University, Melbourne, Australia; 3) Institute for Molecular Biology, University of Queensland, Brisbane, Australia.
The migration of limb and fin myoblasts from their origin in the rostral somites to their ultimate site of differentiation within the limb and fin is a paradigmatic example of a set of dynamic and orchestrates cell migratory behaviours. The physical properties of migrating myoblasts are not well understood. Here we provide the first detailed time-lapse analysis of pectoral fin muscle migration. We developed an optimised confocal imaging protocol by developing custom 3D silicone molds. These molds reduce variation in mounting, reduce z-stack size to a minimum, and maximise temporal resolution. Using these molds and a TgBAC(Pax3a:GFP) that labels muscle progenitors throughout migration we were able to visualize the morphogenetic and physical properties of migrating pectoral fin myoblasts. Contradicting previous work we find somite 4-6 contribute to the zebrafish pectoral fin, while somite 7 contributes to the Posterior Hypaxial Muscle. We find the migratory properties of fin muscle progenitors to both the pectoral fin and the Posterior Hypaxial Muscle resembles 2D sheet migration. This is in contrast to historical work on amniotes, which suggests stream migration as the dominant method employed in limb muscle progenitor migration.

516. A novel zebrafish mutant regulates cellular behavior in the posterior lateral line primordium. ***Hillary F McGraw***[1], *Tor Linbo*[2], *Alex V Nechiporuk*[1]. 1) Cell & Developmental Biology, Oregon Heath & Science University, Portland, OR; 2) Department of Biological Structure, University of Washington, Seattle, WA.
Collective cell migration is important for organ formation in the developing embryo and is often misregulated during invasion of certain cancers. Formation of the posterior lateral line (pLL) mechanosensory system in the zebrafish has proven to be an exquisite model in which to study collective cell migration. The pLL forms from the pLL primordium (pLLp), a cohort of ~100 cells that collectively migrate along the trunk of the zebrafish embryo. The pLLp is comprised of proliferative progenitor cells and organized epithelial cells that will form the mechanosensory organs of the pLL. As part of an ongoing forward genetic screen, we have identify novel mutant, *moon*, which displays defects in patterning of the pLL, including stalling of the pLLp and a failure to form the majority of mechanosensory organs. *moon* mutants are homozygous viable, fertile and grossly behaviorly normal as adults, despite showing several loss of lateral line mechanosensory organs. Analysis of cellular dynamics in the *moon* pLLp revealed a decrease in cellular proliferation. We found that *moon* pLL patterning defects are due in part to a decrease in pLLp proliferation. Although *moon* mutant pLLp do not show a decrease in the proportion of cells that incorporate BrdU, expression of the M-phase marker, phosphohistone H3 is significantly decreased, suggesting that *moon* regulates specific aspects of the cell cycle. In addition, *moon* mutants display a dramatic increase in apoptosis of cells in the migrating pLLp. Expression of factors that are known to regulate cellular proliferation, survival and differentiation in the pLLp, such as members of the canonical Wnt and Fgf signaling pathways are progressively disrupted in *moon* mutant pLLps. Using RNA-sequencing analysis, we found that the lesion unlying the *moon* mutation is located on the distal tip of chromosome 19 and we are currently analyzing candidate genes. The *moon* mutant may provide insight into how distinct cellular behaviors are specifically regulated in the context of a collectively migrating cohort both during normal development and during cancer cell invasion.

517. Mib1 regulates cell migration through negative control of Ctnnd1 function by its ubiqutination. ***T. Mizoguchi***[1], *K. Hirose*[2], *S. Ikeda*[1], *S. Watanabe*[1], *S. Yang*[1], *M. Itoh*[1]. 1) Grad. Sch. of Pharm. Sci., Chiba Univ., Chiba, Japan; 2) Div. of Bio. Sci., Grad. Sch. of Sci., Nagoya Univ., Nagoya, Japan.
The zebrafish posterior lateral line primordium (pLLP) is easily observed and a useful model organ to analyze cell migration. We found that the pLLP migration was delayed in *mind bomb1* (*mib1*) mutants. Mib1 is an E3 ubiqutin ligase and a positive regulator of Notch signal through ubiquitination of Notch ligands. However, Inhibition of other Notch signal components did not cause severe pLLP migration arrest. Therefore, Mib1 could potentially control cell migration via Notch signaling independent pathway. To investigate further the mechanism of Mib1-mediated cell migration, we performed *Mib1* knockdown experiments in HeLa cells. siRNA knockdown of *Mib1* showed an accelerated cell migration compared with control siNRA treated cells. This *Mib1*knockdown effect was suppressed by expression of a siRNA-resistant Mib1 WT, but not by that of an ubiquitin ligase activity deficient Mib1. Moreover, focal adhesion formation was decreased and ectopic protrusions were formed in *Mib1*-knockdown HeLa cell. These data suggest that Mib1 regulates cell migration via substrates involved in cytoskeletal rearrangements other than Notch ligands. Catenin delta 1 (Ctnnd1) is a mediator of cell adhesion, morphology, and motility. A previous study reported that Mib1 could potentially interact with Ctnnd1. Hence, we assessed whether Ctnnd1 is a substrate of Mib1. We found that Mib1 interacted with Ctnnd1 and mono-ubiquitinated it. It is known that Ctnnd1 overexpression induces ectopic protrusions in HeLa cells, but Mib1 suppressed this Ctnnd1-mediated effect in an ubiquitin ligase activity-dependent manner. These data may imply that Mib1 controls cell migration via negative regulation of Ctnnd1 activity. Finally, we investigate functional relationship between Ctnnd1 and Mib1 in zebrafish pLLP migration. We tested the hypothesis that exaggerated Ctnnd1 activity in *mib1* mutant causes the delay in pLLP migration. Morpholino Knockdown of *ctnnd1* partially rescued pLLP migration defects in *mib1* mutants. Taken together, our data suggest that Mib1 is involved in pLLP migration through ubiquitination of Ctnnd1.

518. Non-muscle myosin IIA and IIB differentially regulate cell shape changes in the zebrafish midbrain-hindbrain boundary during morphogenesis. ***Srishti U. Sahu***, *Constance Kwas, Jennifer H. Gutzman.* Department of Biological Sciences, University of Wisconsin Milwaukee, Milwaukee, WI.
Morphological changes in tissue shape during development are formed by specific changes in cell shape. The midbrain-hindbrain boundary (MHB) is one of the first folds in the vertebrate embryonic brain and is highly conserved across species. We used the zebrafish MHB as a model for determining the molecular mechanisms that regulate cell shape changes. Formation of the zebrafish MHB is regulated by specific cell shape changes that occur at the point of deepest constriction, the MHB constriction (MHBC). Cells at the MHBC initially shorten

which occurs between 18 and 22 hours post fertilization (hpf), and by 22 hpf cells at the MHBC are approximately 75% the length of surrounding cells. In addition, cells in the MHB become narrower during this time. Cell shape changes are tightly regulated by signaling pathways that activate and rearrange the cytoskeleton and maintain cell stability. We hypothesized that non-muscle myosin (NM) II proteins were likely mediators of these cell shape changes. We tested this hypothesis using morpholino mediated loss-of-function experiments. We determined that NMIIA (*myh9*) and IIB (*myh10*) are both required for the proper formation of the zebrafish MHB. Additionally, we discovered that NMIIA and IIB function differentially to control cell shape. We found that *myh9* is required for cell shortening, specifically in the cells at the MHBC, while *myh10* is required for the narrowing of the cells throughout the MHB region. Investigation of over-activation of NMII using myosin phosphatase, *mypt1*, loss-of-function further supported the *myh9* and *myh10* knockdown phenotypes. Investigation of actin localization in loss-of-function experiments revealed that loss of NMIIA or IIB resulted in abnormal actin localization. Current experiments are focused on determining the upstream signaling molecules that differentially regulate NMIIA and IIB. Identifying these mechanisms will advance the understanding of the molecular basis for morphogenetic processes during brain formation and are likely to be applicable to developmental events throughout the embryo.

519. CARMIL 2: A novel multidomain scaffold linking actin and vimentin. ***Benjamin C. Stark***, *John A. Cooper.* Dept. of Cell Biology & Physiology, Washington University, St Louis, MO.
Actin assembly is essential for many cellular processes, including cell migration during development and metastasis. Assembly occurs primarily at barbed ends of actin filaments, and the generation of new barbed ends is highly regulated. Capping protein binds to actin barbed ends and stabilizes the filament, preventing loss or addition of actin subunits. CARMIL proteins are a major regulator of capping protein. They are found throughout vertebrates and in some lower eukaryotes including *Acanthamoeba* and *Dictyostelium.* CARMILs bind to and inhibit capping protein via an allosteric mechanism. Most exciting, they are able to remove capping protein from barbed-ends and thereby generate a new, free barbed-end. CARMILs are large, multi-domain proteins. Vertebrate genomes contain three conserved genes that encode CARMILs. Prior to my work, only one CARMIL isoform had been confirmed in zebrafish. I have identified two additional CARMIL genes in the zebrafish genome. All three isoforms of CARMIL in zebrafish are highly conserved with respect to other vertebrates. All three are expressed during development. In human cells, CARMIL 2 co-localizes with vimentin, away from the leading edge and may act as a scaffolding protein between the actin cytoskeleton and intermediate filaments. Morpholino-mediated knockdowns of CARMIL 2 and CARMIL 3 result in isoform-specific phenotypes. CARMIL 2 knockdown results in embryonic lethality in 90% of fish by 24 hpf. CARMIL 3 knockdown results in cardiac defects and death by 5 dpf. Using fish lacking the CARMIL 2 protein, I have begun to study examining the different subdomains of the CARMIL 2 isoform using known, conserved, mutations that perturb specific protein functions: vimentin binding, membrane localization, and capping protein interaction.

520. Limb progenitor cells respond to Fgf convergence cues during pectoral limb bud formation. ***Haley K. Stinnett***[1,3], *Qiyan Mao*[2,3], *Robert K. Ho*[1,2]. 1) Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637; 2) Committee on Development, Regeneration and Stem Cell Biology, University of Chicago, Chicago, IL 60637; 3) Contributed equally to this work.
The zebrafish model greatly facilitates the study of coordinated cell behaviors. In particular, the pectoral limb bud provides a system in which we can both observe cell movement during organogenesis and elucidate the molecular underpinnings of the process. Work in our laboratory has shown that limb progenitor cells are initially arranged in a single layer in the lateral plate mesoderm and then undergo spatio-topic convergence to form a visible limb bud. Fgf24, a zebrafish fibroblast growth factor, is required for proper limb bud formation as first described by Fischer et al. (2003). Based on the function and expression pattern of Fgf24 in the limb field, it has been hypothesized that Fgf24 supplies a convergence cue for oriented limb progenitor cell migration. Our cell tracking experiments show that limb bud progenitors fail to make early directed migrations in Fgf24 morphants, resulting in the absence of pectoral fins. Additionally in this study, we sought to determine if limb progenitor cells respond to, and converge upon, a local source of FGF protein in the lateral plate mesoderm during limb bud formation. We implanted FGF-coated microbeads in the lateral plate mesoderm of Fgf24 morphants. Using 4D live imaging and cell tracking analysis, we found that limb progenitors migrate toward the FGF-coated bead, indicating that Fgf signaling can play a chemotactic role during limb bud formation. This provides further evidence that Fgf24 may indeed act as the convergence cue in the lateral plate mesoderm during pectoral limb bud formation.

521. Regulation of Convergence and Extension by the Myosin Phosphatase Complex during Zebrafish Gastrulation. ***Douglas C. Weiser***. Biological Sciences, University of the Pacific, Stockton, CA.
Vertebrate gastrulation is a tightly regulated series of cellular rearrangements that coordinate the movements of the three germ layers and establishes the embryonic body plan. This process requires a precise regulation of cell polarity, cell migration, and cell division. One of the major driving forces of vertebrate gastrulation is convergent extension, a mechanism in which mesodermal cells move toward the future dorsal side of the embryo and then intercalate between neighboring cells, resulting in an overall dorsoventral narrowing (convergence) and anteroposterior lengthening (extension) of the embryo. Convergence and extension is controlled by a number of signaling pathways, one particularly important pathway is the RhoA-Rock-Myosin pathway that increases actomyosin contractility downstream of the Wnt-PCP pathway. The myosin phosphatase is an important negative regulator of the RhoA-Rock pathway and is itself inhibited by ROCK. Most work in zebrafish has focused on the regulatory subunit of the myosin phosphatase called Mypt1. In this work, we examined the critical role of Protein Phosphatase 1, PP1, the catalytic subunit of the myosin phosphatase. We observed that in zebrafish two paralogous genes encoding PP1 Beta, called ppp1cba and ppp1cbb, are both broadly expressed during early development. Furthermore, we found that both gene products interact with Mypt1 and assemble an active myosin phosphatase complex. In addition, expression of this complex results in dephosphorylation of the myosin regulatory light chain and large scale rearrangements of the actin cytoskeleton. Morpholino knock-down of ppp1cba and ppp1cbb results in severe defects in morphogenetic cell movements during gastrulation through loss of myosin phosphatase function. Our work demonstrates that zebrafish have two genes encoding PP1 Beta, both of which can interact with Mypt1 and assemble an

active myosin phosphatase. In addition, both genes are required for convergence and extension during gastrulation and correct dosage of the protein products is required.

522. Transcriptome analysis identified genes enriched in ocular- or blind-side of flounder. ***H. Yokoi****, X. Wu, M. Kunimasa, Y. Sakai, T. Suzuki.* Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.
Flounder transform their body plan during metamorphosis: right eye migrates to the other side and pigment cells differentiate in a left-right asymmetric manner. It has shown that thyroid hormone signaling triggers the metamorphosis, but little is known about the molecular basis of the morphogenesis during the metamorphosis. Here we performed RNA-seq analysis and compared gene expression profile between ocular-side and blind-side of flounder at post-metamorphosis stage. Approximately 420 million 100-bp reads were obtained and they were mapped onto assembled contigs. More than 30 genes showed 10-fold or higher expression level in ocular side, which include genes known to involve in melanophore differentiation such as *gch* and *mitf*. On the other hand, less than 10 genes showed 10-fold or higher for the blind side. We also present histological distribution of the transcript, which confirmed asymmetric expression between ocular and blind side. These results suggest that more genes are involved in the differentiation of ocular side of body, which is consistent with the asymmetric differentiation of pigment cells restricted in the ocular side.

523. Zebrafish Models of Inherited Muscle Disorders. ***Jane Patrick****, Elisabeth Busch-Nentwich, Derek Stemple.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom.
Inherited muscle disorders comprise a clinically and genetically heterogeneous set of neuromuscular diseases associated with muscle weakness or degeneration. Although the more common causative genes are known many patients have an unidentified rare or unique mutation. This genetic heterogeneity contributes to the lack of therapy: there is currently no cure for these diseases and treatment is limited to management of symptoms. Recently whole exome sequencing of patients with muscular disorders has accelerated the discovery of potential causative mutations. The UK10K project (www.uk10k.org) rare disease sample set includes a number of congenital muscular dystrophy and congenital myopathy cases which have yielded new disease-associated genes. Although the link between mutation and patient phenotype has been established this is generally limited to a descriptive account of the morphological phenotype with little known about the molecular mechanisms involved. We are using the zebrafish as a model to define the molecular phenotypes of these inherited muscle disorders, which have potential to reveal new pathways for therapy. Orthologues of most human muscular dystrophy genes can be identified in the zebrafish genome and the zebrafish has already proved an effective model for both muscular dystrophy and myopathy. We intend to generate a number of knockout zebrafish models and perform phenotypic analysis at the morphological and molecular level. Where available, disruptive alleles already generated by the Zebrafish Mutation Project are used. For the remaining genes targeted genome editing is being carried out using the CRISPR/Cas9 nuclease system. Initial behavioural assays will be followed by a more in-depth investigation at a molecular level. DeTCT (Differential expression Transcript Counting Technique) uses high throughput sequencing to quantitatively analyse changes in transcript levels between mutant embryos and wild-type siblings. Using this approach to identify gene regulatory networks affected by each mutation could give insight into individual disease cases and highlight commonly affected pathways. This molecular analysis has potential to reveal novel avenues for therapy in inherited muscle disorders.

524. Nidogens as novel hemicentin binding partners. ***Stefania Richetti****, Jin Li Zhang, Thomas Ramezani, Julia Hatzold, Matthias Hammerschmidt.* Institute of Developmental Biology, University of Cologne, Cologne, Germany.
The epidermis is separated from the underlying dermis by an extracellular matrix (ECM) structure, the basement membrane (BM). Disruption of the epidermal-BM junction leads to a loss of epidermal integrity, while disruption of the BM-dermal junction causes skin blistering. Constitutive basement membrane components include laminins and Collagen IV, which form two independent networks and are cross-linked by nidogens and Perlecan. We are using the zebrafish caudal fin as a model to understand the role of BM proteins. Mutations in *laminina5* (*lama5*) affect the epidermal-BM junction and lead to a degeneration of the fin fold epidermis as a result of reduced adhesion between keratinocytes. In contrast, mutations in *hemicentin1* (*hmcn1*) cause fin blistering. Hmcn1 is a member of the fibulin family and little is known about its interaction partners. We have tested several candidate ECM proteins for their ability to bind to Hmcn1 by *in vitro* Surface Plasmon Resonance (BIACORE) and found nidogens as the only interacting proteins. Hmcn1 binds to the G3-domain of Nidogen 1 and 2, a domain that also mediates the binding of Nidogen to Laminin. Interestingly, Hmcn1 seems to compete with laminin for Nidogen binding. We have validated a functional interaction between these proteins *in vivo* using morpholino-mediated knockdown approach. Moderately reduced levels of Nidogen 2 or Hmcn1 alone do not result in a morphological phenotype. However, a moderate reduction of both genes simultaneously causes the degeneration of the caudal fin fold, mislocalization of laminins and E-cadherin, and reduced epidermal cell-cell adhesiveness. In conclusion, our data demonstrates a physical and functional interaction between nidogens and Hmcn1, which is required for epidermal homeostasis and point to a new role of Hmcn1 in the epidermal-BM interface.

525. NIM811, a cyclophilin inhibitor devoid of immunosuppressive activity, cures a zebrafish model of collagen VI congenital muscular dystrophy. ***Marco Schiavone***[1]*, Alessandra Zulian*[1]*, Erika Rizzo*[1]*, Elena Palma*[1]*, Francesca Tagliavini*[2]*, Bert Blaauw*[1]*, Luciano Merlini*[2]*, Nadir Mario Maraldi*[2]*, Patrizia Sabatelli*[2,3]*, Paolo Bonaldo*[4]*, Francesco Argenton*[5]*, Paolo Bernardi*[1]. 1) Department of Biomedical Science, University of Padua, Padua, Italy; 2) Laboratory of Musculoskeletal Cell Biology, Istituto Ortopedico Rizzoli, Bologna, Italy; 3) CNR, Institute of Molecular Genetics, Bologna, Italy; 4) Department of Molecular Medicine, University of Padua, Padua, Italy; 5) Department of Biomedical Sciences, University of Padua, Padua, Italy.
Ullrich Congenital Muscular Dystrophy (UCMD) and Bethlem Myopathy (BM) are inherited muscle diseases due to mutations in the genes encoding the extracellular matrix protein collagen (Col) VI. Opening of the cyclosporin A-sensitive mitochondrial permeability transition pore is a causative event in disease pathogenesis, and a potential target for therapy. We have tested the effect of N-methyl-4-isoleucine-cyclosporin (NIM811), a non-immunosuppressive cyclophilin inhibitor, in a zebrafish model of ColVI myopathy obtained by deletion of

the N-terminal region of the Col6a1 triple helical domain, a common mutation of UCMD. Treatment with antisense morpholino sequences targeting col6a1 exon 9 at the 1-4 cell stage (within 1 hour post fertilization, hpf) caused severe ultrastructural and motor abnormalities as assessed by electron and fluorescence microscopy, birefringence, spontaneous coiling events and touch-evoked responses measured at 24-48 hpf. Structural and functional abnormalities were largely prevented when col6a1 zebrafish morphants were treated with NIM811 (which proved significantly more effective and less toxic than cyclosporin A) at 21 hpf. Beneficial effects of NIM811 were also detected in primary muscle-derived cell cultures from UCMD and BM patients, where the typical mitochondrial alterations and depolarizing response to rotenone and oligomycin were significantly reduced; and in the Col6a1-/- myopathic mouse model, where apoptosis was prevented and muscle strength was increased. Since the permeability transition pore of zebrafish shares its key regulatory features with the mammalian pore, our results suggest that early treatment with NIM811 should be tested as a potential therapy for UCMD and BM.

526. What Triggers Zebrafish Fast Muscle Morphogenesis? ***Jared C. Talbot***[1], *Kimberly J. Hromowyk*[1], *David M. Langenau*[2], *Sharon L. Amacher*[1]. 1) Departments of Molecular Genetics and Molecular and Cellular Biochemistry, The Ohio State University, Columbus, OH, USA; 2) Department of Pathology, Molecular Pathology Unit, Massachusetts General Hospital, Boston, MA, USA.
What triggers a muscle cell precursor to rapidly mature into a muscle fiber? In zebrafish embryos, slow-twitch muscle cells (SMCs) mature soon after being specified while fast-twitch muscle cells (FMCs) wait for several hours before they rapidly elongate, and then fuse together into fast muscle fibers. Previous research indicates that FMC activation is triggered by SMCs, and we have identified one FMC marker (EB165) whose expression is delayed in embryos that lack SMCs. However, many other FMC maturation markers do not require SMCs for activation, even though the expression onset of some SMC-independent FMC markers occurs around the time of SMC migration. Hence, we propose that multiple cues work in concert to trigger FMC maturation. To better understand FMC morphogenesis, we are conducting time lapse microscopy of muscle morphogenesis on zebrafish carrying transgenes expressed in SMCs, membranes of FMCs, and muscle nuclei. We are also conducting a CRISPR screen to mutate candidate genes (*brag2, cdh15, ckip1, crk, crkl, kirrel3l, myl1, tmem8c, nhp2l1a, nhp2l1b, nphs1, nr5a2, six1a, six1b, six4a,* and *six4b*) suspected to regulate FMC maturation. Surprisingly, we have found no muscle defects in the first three early-stop mutants examined, *six1a, six1b* and *nr5a2,* contrasting sharply with published morpholino phenotypes. To expand our list of candidate genes and profile genomic mRNA changes during FMC maturation, we will perform RNA-seq on FACS sorted populations (FACS-Seq) from embryos expressing an early FMC marker (*six1b:EGFP*) along with an FMC marker which turns on after muscle elongation (*mylz2:mCherry*). To learn which FMC maturation markers are SMC-dependent, we will compare FACS-seq profiles between WT embryos and embryos lacking SMCs. Our work so far has revealed that multiple pathways govern FMC activation; together, our proposed CRISPR screen, time lapse imaging, and FACS-seq analyses will reveal the molecular nature of cues that trigger FMC activation.

527. Zebrafish modeling of b3GalT6-deficient type of Ehlers-Danlos syndrome stresses the importance of glycosaminoglycans in development. *Tim Van Damme*[1], ***Andy Willaert***[1], *Delfien Syx*[1], *Sofie Symoens*[1], *Suzanne Vanhauwaert*[1], *Sylvie Fournel-Gigleux*[2], *Anne De Paepe*[1], *Fransiska Malfait*[1]. 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) UMR 7365 CNRS-Université de Lorraine (IMoPA), MolCelTEG Team, Biopôle UL, Faculté de Médecine, Vandoeuvre-lès-Nancy, France.
Proteoglycans are important components of cell plasma membranes and extracellular matrices. They are composed of glycosaminoglycan (GAG) chains attached to a core protein through a tetrasaccharide linker region. The addition of the third residue in this linker is catalysed by galactosyltransferase II (b3GalT6), encoded by *B3GALT6*. We recently identified bi-allelic mutations in *B3GALT6* in several individuals from independent families with a severe autosomal recessive pleiotropic connective tissue disorder characterized by skin fragility, delayed wound healing, joint hypermobility and contractures, muscle hypotonia, intellectual disability and a spondyloepimetaphyseal dysplasia with bone fragility and severe kyphoscoliosis. To characterize the function of b3GalT6 we employed zebrafish as an *in vivo* model. Whole mount *in situ* hybridization of zebrafish embryos showed high *b3galt6* expression levels in brain, retina, pharyngeal arches and notochord epithelium, corresponding to tissues that are affected in the human patients. A morpholino-based approach was used to characterize the developmental effects of *b3galt6* knockdown in zebrafish embryos. Complete knockdown of *b3galt6* is lethal, but partial knockdown results in an abnormal pharyngeal cartilage phenotype and a notably reduced head and eye size. These morphological changes were accompanied by a significant reduction in the total amount of sulfated GAG chains. In conclusion, our results emphasize a crucial role for b3GalT6 in GAG synthesis and development. Ongoing and future experiments aim to extensively analyze the changes in GAG composition as well as to further characterize the way in which these changes impact embryonic development of e.g. cartilage and heart using different fluorescent transgenic reporter lines.

528. Structural and functional mapping of zebrafish visual circuits with transsynaptic virus and calcium imaging. ***Yuchin Albert Pan***[1,2], *Clemens Riegler*[2,3], *Kevin Beier*[4,5,6], *Constance Cepko*[3,6], *Florian Engert*[2], *Alexander Schier*[2]. 1) Inst of Mol Med & Genetics, Georgia Regents Univ, Augusta, GA; 2) Department of Molecular and Cellular Biology, Harvard Univ, Cambridge, MA; 3) Dept of Neurobiology, Univ of Vienna, Vienna; 4) Dept of Genetics, Harvard Medical School, Boston, MA; 5) Dept of Biology, Stanford University, Palo Alto, CA; 6) HHMI.
The optical accessibility of the larval zebrafish brain makes it an ideal system to investigate the brain in an intact vertebrate. However, for most neural circuits in zebrafish, the connectivity patterns between brain regions or specific cell types remain unknown. To identify novel neural circuits involved in visual behaviors, we developed a circuit mapping method utilizing genetically engineered vesicular stomatitis virus (VSV) combined with calcium imaging. VSV has recently been found to be capable of infecting rodent neurons and spreading transsynaptically in a manner similar to the rabies virus. VSV, however, is less pathogenic to humans and can be employed for both anterograde and retrograde transsynaptic tracing. We demonstrate that VSV effectively infects zebrafish neurons and show furthermore that it can spread transsynaptically in the anterograde as well as the retrograde direction. We tested anterograde transsynaptic labeling by infecting retinal ganglion cells (RGCs) and examined for viral infection in downstream targets in the diencephalon and midbrain. Longer

viral incubation results in labeling of targets further downstream, like the cerebellum and habenula. To test for retrograde transsynaptic labeling, we infected tectal areas and examined viral infection in RGCs and their presynaptic partners. The patterns of viral spread are consistent with VSV being a transsynaptic tracer in zebrafish. To correlate circuits with function, we infected RGCs that expressed the genetically encoded calcium indicator, GCaMP6f, and showed that VSV-infected neurons responded robustly to specific visual modalities. These results suggest that VSV spreads transsynaptically in zebrafish and the combination of circuit mapping and functional imaging makes it possible to correlate labeled microcircuits with behaviorally relevant visual inputs.

529. Pkd2l1: a TRP channel mediating spontaneous calcium transients in the embryonic spinal cord. ***Andrew Prendergast***[1], *Lydia Djenoune*[1], *Jenna Sternberg*[1], *Arish Shah*[2], *Luyuan Pan*[2], *Cecilia Moens*[3], *Claire Wyart*[1]. 1) Institut du Cerveau et de la Moelle Épinière (ICM) , INSERM UMR 1127, CNRS UMR 7225, UPMC Univ Paris 06, Paris, France; 2) Fred Hutchinson Cancer Research Center, Seattle WA, USA; 3) Institut Curie, CNRS UMR 3215, INSERM U 934, Paris, France.
Polycystic kidney disease 2-like 1 (Pkd2l1) is a non-selective cation channel belonging to the transient receptor potential family. These channels are known to respond to mechanical, chemical, or thermal stimuli. Mouse studies suggest that PKD2L1 is an obligate component of sour taste sensation. In addition to its expression in mammalian taste buds, PKD2L1 is also found in the cerebrospinal fluid-contacting neurons (CSF-cNs) of the spinal cord. CSF-cNs are ventrally-situated GABAergic neurons; apically, they exhibit an extension with intraluminal microvilli in contact with the cerebrospinal fluid, while basally, they project an ipsilateral ascending axonal projection. Previous work has shown that CSF-cNs modulate slow swimming behavior of zebrafish larvae. Recently, we showed that pkd2l1 is highly specific to CSF-cNs in zebrafish at early stages of development. In this study, we investigate the role of this channel in setting the excitability of CSF-cNs. We generated several mutant alleles using engineered nucleases targeting pkd2l1. In mutant embryos and larvae, we do not observe phenotypes associated with ciliary defects such as curvature or edema. We also do not observe any major paralysis. CSF-cNs exhibit spontaneous calcium transients beginning at approximately 22 hpf. This activity is largely uncorrelated (either between CSF-cNs themselves or with spontaneous motoneuron activity) and is restricted to a ventral population of CSF-cNs known as KA'' cells. In pkd2l1 -/- embryos, we observe an almost complete abolition of this spontaneous activity. We are currently investigating the consequences of this loss on the morphology, connectivity and differentiation of CSF-cNs. We are also conducting experiments to examine more subtle aspects of locomotion in the absence of Pkd2l1. Further work will contribute to our understanding of the relevance of this activity on spinal cord development and subsequent sensory-motor integration.

530. The Utility of The Zebrafish Model in Conditioned Place Preference and Novel Tank Diving Test to Assess The Rewarding and Anxiolytic Effects of Mitragynine and Its Analogs. ***Allimalar Sathiaseelan***[1], *Mohd Nizam Mordi*[1], *Surash Ramanathan*[1], *Alexander Chong Shu-Chien*[2], *Sharif Mahsufi Mansor*[1]. 1) Center for Drug Research, University Science Malaysia, Penang, Malaysia; 2) Malaysian Institute of Pharmaceuticals and Nutraceuticals, Malaysian Ministry of Science, Technology and Innovation, Bukit Gambir, Penang, Malaysia.
Mitragynine is the major alkaloid compound found in leaves of Mitragyna speciosa, a plant widely used by opiate addicts to diminish the severity of drug withdrawal. Studies have shown that mitragynine own the ability to suppress withdrawal conditions, together with antinociceptive and antidepressant properties. A series of experiments were conducted to investigate the effects of mitragynine and its analogs on the preference and anxiety-related behaviours using the conditioned place preferences and novel tank diving test in zebrafish. The protocol involves three steps: the determination of initial preference, one conditioning session and the determination of the final preference. This procedure was carried out for a time period of 2 days. In the preference study, after a single drug exposure, morphine, mitragynine and the analogs exhibited preference towards their respective drug-associated compartment at different drug concentrations. In the naloxone antagonist test, morphine demonstrated no preference towards the drug-associated compartment. In the novel tank study, two-week old adult zebrafish were subjected to chronic exposure of morphine. Then, the zebrafish were placed in a water system (24 hours) for withdrawal observation. In this test morphine withdrawn zebrafish displayed anxiety-related swimming behaviors such as decreased exploratory behavior and increased erratic movement. Interestingly, upon exposure to mitragynine and its analogs, the withdrawn zebrafish showed a significant reduction in stress-related swimming behaviours respectively. This observation suggests that mitragynine and its analogs alleviate opiate withdrawal behaviours in zebrafish.

531. Serotonin system function in nicotine-induced locomotor behavior. ***H. Schneider***, *J.M. Abarr, C.A. O'Brien, B.F. Kopecky, D.R. Brueck, N.J. Snyder, S. Owiredu, E.E. Clor, S. India-Aldana, K.Y. Chen, Y. Liu, E.M. Buening.* Department of Biology, DePauw University, Greencastle, IN.
The serotonin system has been linked to the modulation of psychostimulant-induced behavior and could be trageted for the treatment of nicotine dependence, the number one cause of preventable diseases in the U.S. Measuring behavioral responses of zebrafish larvae to nicotine following treatment with serotonin receptor agonists could lead to identification of potential new chemicals for smoking cessation therapy in humans. The zebrafish neurobehavioral phenotype assay provides an alternative approach for screening chemicals effectively. Results presented here address the role of serotonin receptors htr1a, htr1b, and htr7 in modulating nicotine-induced locomotor response behavior and are compared with actions of varenicline, the active chemical in the smoking cessation drug Chantix. Neurobehavioral studies show that both htr1a and htr1b agonists reduce the spontaneous swimming activity and the actute locomotor response to nicotine. However, the reduction of the nicotine-induced locomotor activity is not specific. Studies using htr7 agonists indicate that activation of htr7 reduces the behavioral response to nicotine. The effectiveness of htr7 agonists varied. However, all tested agonists reduced nicotine-induced locomotor activity non-specifically after a 24 hr incubation. Genes of tested serotonin receptors appear to be active in larvae. The neurobehavioral studies indicate that htr1a, htr1b and htr7 do not seem to be involved in a specific regulation of nicotine-induced locomotor behavior in larval zebrafish. The effects of tested serotonin receptor agonists and varenicline on nicotine-induced locomotor behavior don't match in larval zebrafish. Thus, the tested chemicals seem less likely to represent good targets for treatment of nicotine dependence.

532. Gene expression profiling of the zebrafish interpeduncular nucleus. ***Abhignya Subedi***[1,2], *Erik Duboué*[1], *Marnie Halpern*[1,2]. 1) Embryology, Carnegie Institution for Science, Baltimore, MD; 2) Biology, Johns Hopkins University, Baltimore, MD.
The interpeduncular nucleus (IPN) is a midline structure of the ventral midbrain that is part of a highly conserved neural pathway connecting forebrain nuclei with the midbrain. Efferents from neuronal populations in the left and right dorsal habenular nuclei (dHb) terminate in differing regions of the IPN, suggesting that the IPN is functionally specialized along its dorsoventral axis. In support of this, we carried out transcriptional profiling of the IPN and identified genes that show expression in discrete subregions. The transgenic line *TgBAC(gng8:Eco.NfsB-2A-CAAX GFP)*^{c375} robustly and selectively labels dHb neurons and their axons with membrane tagged GFP in both the larval and adult brain. Using the GFP positive axon terminal bundles as a guide, we microdissected the IPN from adult zebrafish brains and pooled tissues for RNA extraction and transcriptional profiling. In replicate experiments, 406 transcripts were found to be significantly enriched in the IPN sample compared to the rest of the adult brain tissue. We assayed 30 genes by RNA in situ hybridization to determine whether they were expressed in the IPN at larval as well as adult stages. Notably, *somatostatin 1.1 (sst1.1)* is expressed in cells throughout the IPN, whereas somatostatin 3 (sst3) transcripts are restricted to the ventral subnucleus. Transcripts for some genes are enriched in an intermediate region that separates the dorsal and ventral IPN. In addition to characterizing IPN gene expression, we produced a transgenic line, *Tg(sst1.1:mCherry-CAAX)*^{c459} in which *sst1.1* regulatory sequences drive expression of membrane-tagged mCherry in a subset of dHb neurons. The mCherry labeled dHb neurons project to a distinct subregion of the ventral IPN, demonstrating the specialization of afferent input along the dorsoventral axis of the IPN. These studies provide a framework for characterizing the neuronal subpopulations of the poorly understood IPN, their patterns of connectivity and ultimately their precise functions.

533. Direct activation of the Mauthner cell by electric field pulses drives ultra-rapid escape responses. ***Kathryn Tabor***[1], *Sadie Bergeron*[1], *Eric Horstick*[1], *Diana Jordan*[1], *Vilma Aho*[2], *Tarja Porkka-Heiskanen*[2], *Gal Haspel*[3], *Harold Burgess*[1]. 1) NICHD, NIH, Bethesda, MD; 2) Institute of Biomedicine, University of Helsinki, Helsinki, Finland; 3) Department of Biological Sciences, New Jersey Institute of Technology, Newark, NJ.
Rapid escape swims in fish are initiated by the Mauthner cells (M cells), giant reticulospinal neurons with specializations for swift responses. M cells directly activate motoneurons and facilitate predator detection by integrating acoustic, mechanosensory and visual stimuli. In addition, larval fish show well-coordinated escape responses when exposed to electric field pulses (EFPs). To assess the role of the M cell in the EFP response we enhanced its membrane excitability by overexpression of the voltage-gated sodium channel SCN5. This novel strategy allows us to examine if a neuron contributes to a behavioral response under normal assay conditions. Sensitization of the M cell increased EFP responsiveness suggesting the M cell initiates this escape response. To determine if M cells are required for EFP responses we engineered a variant of nitroreductase with increased activity (epNTR) to selectively ablate M cells. After ablation the response was lost demonstrating that M cells are necessary for the EFP response. The reaction time to EFPs is extremely short, with many responses initiated within 2 ms of the EFP. Large neurons, such as M cells, show heightened sensitivity to extracellular voltage gradients. We therefore tested if the rapid response to EFPs was due to direct activation of M cells, bypassing delays imposed by stimulus detection and transmission by sensory cells. Consistent with this, calcium imaging using GCaMP6s indicated that EFPs robustly activated the M cell, but only rarely fired other reticulospinal neurons. Furthermore, pharmacological block of synaptic transmission did not affect M cell activity in response to EFPs. Moreover, M cells transgenically expressing a tetrodotoxin (TTX) resistant voltage-gated sodium channel retained responses to EFPs despite TTX suppression of action potentials in the rest of the brain. Surprisingly, EFPs directly activate M cells due to their large size, thereby driving ultra-rapid escape responses in fish.

534. The study of the hippocampal function in zebrafish. ***Hideyuki Tanabe***[1], *Pradeep Lal*[1,2], *Akira Muto*[1,2], *Koichi Kawakami*[1,2]. 1) Molecular and Developmental Biology, National Institute of Genetics; 2) Department of Genetics, The Graduate University for Advanced Studies (SOKENDAI).
Hippocampus is part of the limbic system in mammals, and plays an important role in learning and memory. To elucidate cellular and molecular basis for learning and memory, we aim to study hippocampal functions in zebrafish. First, we developed a trace two-way active avoidance conditioning system in zebrafish. In this system, green light is given as the conditioned stimulus (CS), and then an electrical shock is given as the unconditioned stimulus (US) after a temporal gap between CS and US. It has been thought that, the hippocampal function is essential to perform avoidance response in the trace conditioning paradigm. Using this assay system, we demonstrated that wild type zebrafish learn active avoidance in response to CS. Next, we have performed enhancer trap and gene trap screens, and isolated transgenic zebrafish, which had Gal4 expression in specific regions of the adult brain. We crossed them with the UAS:neurotoxin lines, inhibited neuronal activity in the specific regions and analyzed the double transgenic fish using the behavioral assay system. We found transgenic fish, which expressed Gal4 in the dorsolateral telencephalon (Dl), showed impairment of avoidance learning in the trace conditioning when crossed with UAS:neurotoxin. From this study and previous anatomical studies, tDl of zebrafish may be functional equivalent of the mammalian hippocampus.

535. A single mutation in the acetylcholine receptor delta-subunit causes distinct effects in two types of neuromuscular synapses. ***Tory Williams***[1], *Jee-Young Park*[1], *Meghan Mott*[1], *Hiromi Ikeda*[1], *Hua Wen*[2], *Michael Linhoff*[2], *Fumihito Ono*[1]. 1) Laboratory of Molecular Physiology, National Institute on Alcohol Abuse & Alcoholism, Rockville, MD; 2) Oregon Health and Science University, Portland, OR.
Mutations in acetylcholine receptor (AChR) subunits, expressed as pentamers in neuromuscular junctions (NMJ), cause multiple types of congenital myasthenic syndromes. In AChR pentamers, the adult e subunit gradually replaces the embryonic g subunit as the animal develops. Due to this switch in subunit composition, mutations in some subunits result in synaptic phenotypes that change with developmental age. However, a mutation in any subunit is considered to affect the neuromuscular junctions of all muscle fibers equally. Here, we report a zebrafish mutant of the AChR d subunit that exhibits two distinct NMJ phenotypes specific to two muscle fiber types: slow or fast. Homozygous fish harboring a point mutation in the d subunit form functional AChRs in slow muscles, while receptors in fast

muscles are non-functional. To test the hypothesis that different subunit compositions in slow and fast muscles cause distinct phenotypes, we used Transcription Activator-Like Effector Nuclease (TALEN) technology to knock out the function of the gene encoding the e subunit in zebrafish. Larvae homozygous for the e subunit gene mutation expressed AChR synapses only in slow muscles after the g- e switch, which suggests that AChRs in slow muscles do not contain the g/e subunit. This finding in zebrafish suggests that some mutations in human congenital myasthenic syndromes may affect slow and fast muscle fibers differently.

536. *Prdm12b* specifies the p1 progenitor domain of the ventral neural tube and reveals a role for V1 interneurons in swim movements. ***Denise A. Zannino***[1]*, Gerald B. Downes*[2]*, Charles G Sagerström*[1]. 1) Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, MA; 2) Department of Biology, University of Massachusetts, Amherst, MA.
The dorsoventral (DV) axis of the neural tube is divided into 11 domains, each expressing a unique transcription factor code and giving rise to a unique cell type. For example, V1 interneurons (IN) arise from the p1 domain while motor neurons and oligodendrocytes share origins in the pMN domain. Perturbation in DV patterning leads to miss-specification, or loss, of particular cell types. We discovered a relatively uncharacterized gene, *prdm12b*, with expression limited to the p1 domain. Prdm12b is a putative transcription factor belonging to a class of genes characterized by a Pr containing domain and multiple zinc fingers. To determine the role of *prdm12b* in DV patterning, we used antisense morpholino oligonucleotides (MO) designed to block the translation of Prdm12b. We find that loss of *prdm12b* function leads to loss of V1 INs, identified by *eng1b* expression. Loss of V1 INs also leads to deficits in touch evoked escape responses, as these INs play inhibitory roles in locomotion circuits. Specifically, control larval embryos touched on the head arch their bodies into a single c-bend, bringing the head adjacent to the tail, thus orienting the head away from the stimulus, and then swim away in a stereotypical manner. MO-injected embryos exhibit more c-bends, longer durations of response, and swim shorter distances than control MO-injected embryos. Additionally, the left-right alternation of body bends appears delayed. Loss of *prdm12b* function also leads to misexpression of the DV markers *nkx6.1* and *pax3*, expressed respectively ventrally and dorsally to *prdm12b*, as well as to a reduction of *olig2* expression in the pMN domain of the hindbrain. The hindbrain reduction of *olig2* leads to an early reduction in oligodendrocyte lineage cells. Taken together, *prdm12b* regulates DV patterning, is required for V1 IN specification, and plays an important role in escape responses.

537. Interactions of Environmental Neurotoxins with SOD1 in Amyotrophic Lateral Sclerosis in a Zebrafish Model. ***Roger Sher****, Samantha Powers, Emily Lovejoy, Samantha Kwok, Thomas Lavin.* Molecular and Biomedical Sciences, University of Maine, Orono, ME.
Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease that affects upper and lower motor neurons, leading to progressive paralysis, muscle atrophy, and eventually death, with a median survival of 2-5 years. Both genetic and environmental causes of this disease have been identified, but how they lead to a disease mechanism is currently unknown. Most cases of ALS (~90%) are sporadic (SALS), with environmental neurotoxins being epidemiologically associated with development of the disease. Approximately 10% of ALS is familial (FALS), with ~20% of FALS resulting from mutations in the Cu/Zn superoxide dismutase (SOD1) gene. The large variation seen in onset and progression of ALS (both SALS and FALS) is likely due to differing genetic susceptibilities and environmental exposures during development. By determining cellular pathways are involved in modifying neurological defects, both by toxicants and genetic influences, we hope to gain a better understanding of the root causes of this disorder, and to decipher the cellular pathways that lead to neuronal death. The zebrafish, Danio rerio, has been shown to be a robust model organism for modeling human neurodegenerative diseases, including ALS. Our research aims to study the intersection of environmental neurotoxins on motor neuron defects in a zebrafish model of ALS. We are presently determining the impact of BMAA (b-methylamino-L-alanine) on neurological defects and neurodegeneration in mutant SOD1-ALS zebrafish. We have found that (1) 30hpf motor neuron growth and (2) 72hpf neuromuscular junction architecture are significantly altered in SOD1-mutant but not in wild type embryos when exposed to low doses of BMAA. We are presently assessing the progression of neurological degeneration in these fish as they age after this initial embryonic exposure regimen.

538. Microglial depletion reduces neurogenesis in response to QA-induced lesioning in adult zebrafish telencephalon. ***Kaia Skaggs***[1]*, Daniel Goldman*[2,3,4]*, Jack Parent*[1,4]. 1) Neurology, University of Michigan, Ann Arbor, MI; 2) Biological Chemistry, University of Michigan, Ann Arbor, MI; 3) Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI; 4) Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI.
Zebrafish represent an attractive system for the study of neurogenesis following injury because, unlike mammals, they regenerate damaged neurons. We developed a novel brain injury model using quinolinic acid (QA) excitotoxic telencephalic lesioning in adult zebrafish to study neural regeneration. After lesioning, initial cell death is followed by robust inflammation 1-2 days post-injury and a marked increase in proliferation or radial glia-like progenitors that peaks 3-4 days post-injury. The neural progenitors give rise to increased numbers of newborn neurons that migrate to injury and integrate, likely contributing to repair of the lesioned brain. In order to study the role of the early inflammatory response on neurogenesis, we ablated microglia that responded to injury though injection of liposomes containing Clodronate at the time of lesioning. Proliferation and neurogenesis were markedly reduced following Clodronate treatment. These effects persisted over time, resulting in incomplete repair in Clodronate-treated, lesioned brains compared to control liposome-treated, lesioned brains. These results indicate that the early inflammatory response following QA-induced excitotoxic lesioning is an important signaling event that stimulates neurogenesis and repair of adult zebrafish telencephalic brain injury.

539. Wnt/b-catenin Signaling is Necessary for Zebrafish Spinal Cord Regeneration. ***Nicholas S Strand***[1]*, Timothy A Petrie*[2]*, Randall T Moon*[1,2]. 1) Pharmacology, University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute and the Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA.
The Wnt/b-catenin signaling pathway has been shown to be necessary for regeneration in several contexts, across multiple species and various tissues. Wnt/b-catenin has also been shown to be necessary for neural development across vertebrate species, including axis formation, dorsal/ventral patterning, and progenitor fate. The adult zebrafish (Danio rerio) is capable of regenerating motor neurons and

regaining full motor activity after spinal cord injury, making it an interesting model for neuronal regeneration studies. To determine if Wnt/b-catenin signaling is involved in zebrafish spinal cord regeneration, transgenic Tg(7xTCF-xlaSiam:nlsmCherry)ia5 fish were used to report endogenous b-catenin activity after complete spinal cord transection. We found that b-catenin is upregulated 3 days after injury; persists through 14 days, the timepoint of motor neuron proliferation and differentiation; and returns to basal levels by 42 days post-transection. We then inhibited Wnt/b-catenin signaling by inducing Dkk1 via the transgenic fish Tg(hsp70l:dkk1-GFP)w32. Dkk induction and subsequent Wnt/b-catenin inhibition led to a significant decrease in locomotor recovery, assessed via swim score and free swimming recovery. Dkk induction also led to a significant decrease in microglia accumulation to the injury site. It has been previously published that microglia accumulate in a pattern similar to what we have observed in b-catenin responsive cells; however, colocalization studies show that microglia are not directly responding to Wnt/b-catenin signaling. These findings demonstrate that Wnt/b-catenin signaling is necessary for zebrafish spinal cord regeneration and suggest a novel role for Wnt/b-catenin signaling in microglial recruitment in zebrafish.

540. Innervation regulates synaptic ribbons in mechanosensory hair cells. ***Arminda Suli***[1], *Remy Pujol*[2], *Dale Hailey*[3], *Edwin Rubel*[4], *David Raible*[3]. 1) Physiology and Developmental Biology, Brigham Young University, Provo, UT; 2) Inserm, Montpellier, France; 3) University of Washington, Department of Biological Structure, Seattle, WA; 4) University of Washington, Bloedel Hearing Research Center, Seattle, WA.
Hearing and balance relies on the ability of specialized mechanosensory cells, known as hair cells (HC), to function properly. Mechanical activation of HCs causes generation of an electrical signal, which is transmitted to afferent neurons through synapses called ribbon synapses. Ribbons are electron dense structures that tether synaptic vesicles at the active zone, juxtaposed to afferent fibers. To study ribbon synapse formation in HCs, we use the more accessible zebrafish lateral line sensory system, which is used to detect prey and predators. HCs in this system are similar in structure and function to auditory and vestibular HCs. We have done a careful analysis of ribbon dynamics during HC regeneration both by immunohistochemistry and electron microscopy. We find that ribbon formation and maturation during regeneration is very similar with its dynamics during development. Furthermore, by looking at mutants that lack HC innervation or wild-types in which the nerves have been transacted, we find that innervation is critical for regulation of ribbon size, number and localization at the synapse. We are currently determining if physical contact or activity with afferent or efferent innervation is responsible for ribbon regulation.

541. TALENs and CRISPR/Cas models of human metabolic diseases in zebrafish. ***K Tuschl***[1,2], *LE Valdivia*[2], *PT Clayton*[1], *PB Mills*[1], *SW Wilson*[2]. 1) Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, United Kingdom; 2) Department of Cell and Developmental Biology, University College London, United Kingdom.
Purpose: Next generation sequencing is increasingly providing candidate genes associated with human disease. Hence, it is imperative to uncover the role of these genes to understand disease pathogenesis. In our department of paediatric metabolic medicine we study disorders of manganese (Mn) homeostasis leading to deposition of Mn in the basal ganglia associated with dystonia/Parkinsonism. To date, the mechanisms underlying Mn toxicity are poorly understood and treatment options are scarce. We have chosen zebrafish, an experimentally tractable organism, to model inherited metabolic disorders using TALENs and CRISPR/Cas genome editing. Methods and Results: The zebrafish orthologue of the human Mn transporter gene SLC30A10 was cloned and sequence verified. A second transcript utilising an alternative first exon was identified. RT-PCR showed that SLC30A10 is expressed throughout embryonic and larval development. Whole mount in situ hybridization detected SLC30A10 expression in liver, intestine and pharynx; this expression pattern is similar to that seen in human. Yeast auxotrophy experiments confirmed for both the human and zebrafish SLC30A10 protein to play a role in Mn transport. TALENs or CRISPR guide RNAs were designed to target highly conserved regions within the gene and injected into single cell embryos. Restriction enzyme digestion or high resolution melting analysis detected a high rate of somatic mutations at one day post fertilisation. Disruptive mutations were efficiently transmitted to the progeny and carriers harbouring frame-shift indels selected for functional studies. Simultaneous injection of two guide RNAs targeting two different exons generated large deletions spanning more than 11.5 kb thereby preventing rescue of function by alternative transcripts. Conclusions: We have generated a zebrafish loss of function model for a Mn transporter gene using TALEN and CRISPR/Cas methods. This model will provide a platform to better understand the mechanisms of Mn homeostasis, allow the identification of novel therapeutic targets and provide a route for drug discovery.

542. Identification of transcription factors driving GAP-43 gene expression and optic nerve regeneration in zebrafish. ***Ishwariya Venkatesh***, *Ava Udvadia*. Biological Sciences, University of Wisconsin, Milwaukee, Milwaukee, WI.
Disruption of neuronal networks due to injury in the central nervous system (CNS) of adult mammals results in permanent disability. In contrast, fish are capable of CNS regeneration leading to full functional recovery. The ability to regenerate is driven by the capacity of fish to respond to CNS injury with the re-expression of key neuronal growth-associated genes. However, our knowledge of the regulatory mechanisms driving growth-associated gene expression during successful CNS regeneration in fish remains limited. Understanding pathways driving successful CNS regeneration in fish will allow us to target similar pathways to improve regeneration in mammals. Neuronal Growth-associated protein-43 (GAP-43) is highly expressed during CNS development and regeneration, and our studies demonstrate that knockdown of gap43 gene expression during optic nerve injury in fish reduces the rate of regenerative axon outgrowth in vivo. Through phylogenetic footprinting analysis, we identified putative transcription factor binding sites within regeneration specific gap43 promoter regions. A subset of these transcription factors had established roles in axon growth and guidance and hence we hypothesized that they may be involved in regulating regenerative gap43 expression in fish. Using in vivo reporter assays and quantitative PCR, we have identified select transcription factors that are required for regenerative gap43 expression. We have optimized conditions for carrying out chromatin immunoprecipitation (ChIP) on regenerating zebrafish retina and are presently carrying out experiments to determine whether candidate transcription factors regulate gap43 by directly binding to promoter regions in vivo. We are also simultaneously analyzing the effects of transcription factor knockdown on optic regeneration in vivo through retrograde labeling of

regenerating axons from the optic tectum. These studies have identified transcription factors required for successful reactivation of gap43 gene and optic nerve regeneration in zebrafish and enhance our understanding of molecular pathways driving successful vertebrate CNS regeneration.

543. High-throughput neurobehavioral phenotyping in zebrafish models of Parkinsonism. ***Yangzhong Zhou***[1], *Qing Bai*[1], *Edward A. Burton*[1,2,3,4,5]. 1) Pittsburgh Institute for Neurodegenerative Diseases, Department of Neurology, Pitt, PA; 2) Department of Microbiology and Molecular Genetics, Pitt, PA; 3) Department of Neurology, VA Pittsburgh Healthcare System, PA; 4) Geriatric Research, Education and Clinical Center, VA Pittsburgh Healthcare System, PA; 5) Division of Movement Disorders, Pitt, PA.
Parkinson's disease (PD) is a highly prevalent akinetic-rigid movement disorder, characterized by degeneration of CNS dopamine neurons. The overall goal of our work is to develop neuroprotective therapies by exploiting the high-throughput capabilities of zebrafish models for drug discovery. One potential advantage of carrying out these studies in vivo is the possibility of using neurobehavioral assays as endpoints to evaluate dopaminergic function. This might be advantageous over assays relying on detection of cell death since motor function should be sensitive to early pathological changes in nerve terminals, prior to the onset of cell death at the point in pathogenesis when neuroprotective approaches are expected to be efficacious. Furthermore, unlike evaluation of specific biochemical targets, motor assays can be carried out without preconceptions regarding mechanism, potentially allowing truly unbiased identification of neuroprotective molecules. High-throughput, low-cost neurobehavioral profiling of larval zebrafish can be carried out easily in 96-well plates by videographic analysis. In order to develop screening assays suitable for exploitation of zebrafish PD models, we have analyzed aspects of zebrafish motor function that: (i) rely on dopaminergic function; and (ii) can be measured in 96-well plates. We have developed methodology with optimized signal:noise characteristics for quantification of larval motor performance and determined how larval zebrafish motor functions are altered by exposure to the dopaminergic neurotoxin MPP+. Abnormalities of dopaminergic function provoke stereotyped and reproducible changes in zebrafish motor behavior. These data, in combination with complementary parallel studies focused on the development of zebrafish Parkinson's disease models, provide essential resources for in vivo chemical screens aiming to identify novel neuroprotective agents in Parkinson's disease.

544. A DICER-miRNA interaction controls the time course of neurogenesis. ***E. Ristori***[1], *M.A. Lopez-Ramirez*[1], *A. Narayanan*[1], *G. Hill-Teran*[1], *C. Calvo*[2], *J.L. Thomas*[1,2], *S. Nicoli*[1]. 1) Yale Cardiovascular Research Center, Internal Medicine, Yale University, New Haven, USA; 2) University Pierre et Marie Curie-Paris 6, Institut du Cerveau et de la Moelle Epiniere, UMR S1127, Paris, France.
The homeostatic mechanisms that govern the cell type-specific accumulation of miRNAs remain poorly understood. These mechanisms are especially important to drive cell differentiation processes, like neurogenesis. The RNAse enzyme, DICER, operates in vitro in a dose and substrate selectivity-manner resulting in preferential biogenesis of specific miRNAs. Whether the control of DICER expression level is mandatory for the precise miRNA, accumulation in differentiating neural cells in vivo is unknown. Here we found that dicer expression levels are under the post-transcriptional control of miR-107 during zebrafish neural cell differentiation. We revealed that endogenous miR-107 and dicer transcripts have opposite temporal expression patterns in differentiating neuronal cells. Furthermore we showed that TALEN MIR-107 mutant embryos and cultured adult zebrafish neurospheres, in absence of miR-107 manifest high levels of dicer in post-mitotic neural cells. We demonstrated that a direct miR-107-dicer interaction preferentially regulates biogenesis of miR-9 up to a specific threshold that controls properly the time-course of neuron production. The accumulation of miR-9 in neural differentiating cells results in excessive neurogenesis. We found that the signaling involved includes miR-107-dicer-miR-9-Notch target her6-proneural neurog-1 and that this pathway is conserved during embryonic and adult neurogenesis, providing the first evidence that the feedback interaction between miR-107s and DICER is indispensable throughout life. The miR-107-dicer interaction is therefore a previously unrecognized regulatory mechanism required to precisely maintain the specific homeostatic miRNA levels during embryonic and adult neurogenesis.

545. Multiple roles for Wnt signaling in habenular development. ***Sara Roberson***[1,2], *Yung-Shu Kuan*[3], *Lea Fortuno*[1], *Courtney Akitake*[1], *Joshua Gamse*[4], *Cecilia Moens*[5], *Marnie Halpern*[1,2]. 1) Embryology Dept, Carnegie Institution for Science, Baltimore, MD; 2) Biology Dept, Johns Hopkins University, Baltimore MD; 3) Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan; 4) Dept of Biological Sciences, Vanderbilt University, Nashville, TN; 5) Fred Hutchinson Cancer Research Center, Seattle, WA.
The habenulae are bilaterally paired nuclei in the epithalamus that have been implicated in a variety of behaviors such as fear and addiction. Despite their importance in modulating other neural pathways, our knowledge of how the habenulae develop is limited. The zebrafish habenulae consist of dorsal and ventral nuclei, with the former showing prominent left-right (L-R) differences in their size, organization and connectivity. Previously, Wnt signaling was found to be important for this L-R asymmetry and for the development of the ventral habenulae. We now describe an additional requirement for the Wnt pathway in the generation of the dorsal habenulae. We isolated a mutation in the zebrafish homolog of *wntless* (*wls*) from a mutagenesis screen to identify genes involved in habenular development and L-R asymmetry and recovered a second allele by TILLING. Wls is a transmembrane protein required for the secretion of Wnt proteins and is therefore thought to be essential for Wnt signaling. Homozygous *wls* mutants undergo normal early development, but later have small otic vesicles, reduced jaw and fin cartilages, fail to develop a swim bladder and die by 12 days post fertilization. Mutants also form small dorsal habenular nuclei and lack ventral habenulae. However, in contrast to other Wnt signaling mutants, L-R asymmetry of the dorsal habenulae is intact and, outside of the epithalamus, patterning of the brain appears normal. To determine the cause of the small habenulae phenotype, we examined habenular precursors and found a reduction in their number in *wls* mutants. We propose that in addition to its roles in L-R asymmetry of the dorsal nuclei and in the formation of the ventral habenulae, Wnt signaling is also an important regulator of dorsal habenular precursors.

546. Integration of Fgf and RA signaling in the hindbrain by Hox paralog group 1 genes. ***Lyndsay G Selland****, Andrew J Waskiewicz.* University of Alberta, Edmonton, Canada.
During vertebrate development, the hindbrain forms transient rhombomere segments that each contain a unique population of neurons. Identity of hindbrain neurons is regulated by rhombomere-specific gene expression. The combined activities of the Fibroblast growth factor (Fgf) and Retinoic Acid (RA) signaling pathways specify rhombomere 4 (r4), an embryonic organizer in the centre of the hindbrain. *Hox-1* paralog genes are expressed early during development within the presumptive r4. Our goal is to identify the genetic pathways linking Hox-1 paralogs with Fgf and RA signaling. We have created a novel *hoxb1b* mutant via TALEN mediated mutagenesis and combined it with a *hoxb1a* mutant to create a complete loss of Hox-1 paralogs in the hindbrain. We are examining the effect of the loss of these genes on the development of the r4 signaling center and subsequent hindbrain development. *Hoxb1a/b* mutants have severe morphological defects and are not viable. Additionally, the loss of Hox-1 paralogs causes defects in both cranial motor and reticulospinal neuron specification. To begin to define the interactions between Hox, Fgf and RA in the hindbrain, we examined *fgf3* and *fgf8a* expression in *hoxb1a/b* mutants. *Fgf3* is completely lost from r4 in *hoxb1a/b* mutants and the expression domain of *fgf8a* is significantly altered. To examine the contribution of RA we assessed *hnf1ba*, which is activated by RA. *Hnf1ba* shows a strong reduction in *hoxb1a/b* mutants suggesting a reduction in RA signaling. Fgf and RA signaling specify multiple downstream hindbrain patterning genes. Expansion of *krox20/egr2b* expression in r3 in *hoxb1a/b* mutants is indicative of a reduction in Fgf signaling. Additionally, Fgf signaling in combination with *hnf1ba* synergize to initiate *krox20/egr2b* expression in r5, a process that is aberrant in the *hoxb1a/b* mutant. *Mafba* is also activated by *hnf1ba* in combination with Fgf signals from r4, and shows a reduced expression domain in *hoxb1a/b* mutants. In light of these interactions in the *hoxb1a/b* mutant, we postulate a model wherein *hoxb1a* and *hoxb1b* lie atop a regulatory hierarchy of signaling molecules controlling hindbrain development.

547. A functional study of the endocannabinoid system during zebrafish neural development. *A. Martella[1], **R.M. Sepe**[2], L. Ippolito[2], E. De Felice[2], C. Silvestri[1], O. Carnevali[3], P. Sordino[2,4], V. Di Marzo[1]*. 1) Endocannabinoid Research Group, Institute of Biomolecular Chemistry, CNR, Pozzuoli, Italy; 2) Laboratory of Cellular and Developmental Biology, Stazione Zoologica Anton Dohrn, Naples, Italy; 3) Department of Marine Science, Università Politecnica delle Marche, Ancona, Italy; 4) Institute for Mediterranean and Forestal System, Catania, Italy.
The endocannabinoids (eCBs), anandamide (AEA) and 2-arachidonyl glycerol (2-AG), are retrograde neurotransmitters that modulate synaptic transmission in the adult brain. It has been suggested that the presence of eCBs, the CB1 receptor (*Cnr1*) and metabolizing enzymes, during embryogenesis, is linked to different neural developmental processes. Zebrafish possesses the complete endocannabinoid system (ECS). To understand its activity during development, we analyzed ECS genes and endogenous ligands at different embryonic stages. qPCR analyses revealed that the expression of the enzymes of AEA synthesis (*Abhd4, Nape-pld*) and degradation (*Faah*), of the 2-AG anabolic enzyme (*Dagla*), of the *Cb1, Trpv1* and *Gpr55* receptors, increases during development, while 2-AG hydrolyzing enzymes (*Mgll, Ptgs2b*) are downregulated at 48 hpf. Next, we focused on endogenous ligands, revealing that 2-AG is the most abundant in the developing embryo and its levels mirror the expression profile of its synthesis enzyme. The enzymatic assays of DAGLa and MGLL showed that their activity increases during embryogenesis. Moreover, we analyzed the expression profile of *Mgll* during zebrafish neural development by *in situ* hybridization, revealing regions of unique and overlapping expression with *Dagla* and *Cb1*. We pointed out the importance of the 2-AG signaling in CNS development by morpholino knockdown of *Dagla* and *Mgll*, resulting in defective axonal growth and fasciculation, with disruption of axon guidance. Our study shows that the zebrafish ECS is able to actively produce endocannabinoids during embryogenesis. It suggests that the development of correct neuronal circuitries in zebrafish brain relies on the fine control of the 2-AG synthesis and hydrolysis, providing evidence for its key role during neurogenesis, acting as mediator for axonal pathfinding and fasciculation.

548. Functional analysis of de novo DNA methylation and demethylation during zebrafish eye development. ***Pawat Seritrakul****, Jeffrey Gross.* Molecular Biosciences, University of Texas at Austin, Austin, TX.
DNA methylation is an epigenetic mechanism by which methyl groups are added to DNA strands predominantly at cytosine bases (5mC) of gene promoters, and which has been shown to often inversely correlate with gene transcription levels. De novo DNA methylation, mediated by dnmt3-family proteins, adds new methylation marks to regions of hypomethylated DNA, an event thought to be involved in cellular differentiation. Demethylation, initiated by tet-family of dioxygenases, removes existing methylation marks by converting 5mC to 5-hydroxymethylcytosine (5hmC). Little is known about the role of de novo methylation and demethylation during the regulation of tissue-specific development of complex organs like the eye. The zebrafish retina is composed of neuronal and glial cells that are derived from a common pool of seemingly indistinguishable retinal progenitor cells. The zebrafish lens is a unique structure consisting of a proliferative lens epithelium that continuously gives rise to differentiated lens fiber cells in the lens cortex. Given their unique architectures and developmental programs, the zebrafish lens and retina serve as ideal structures in which to study the epigenetic regulation of cellular differentiation. Gene expression analyses revealed that all six de novo methyltransferases (dnmt3-8) are expressed in distinct but overlapping domains in the eye. Additionally, tet2 and tet3 are expressed in the ganglion cell layer and inner nuclear layer of the retina, resembling dnmt6 and dnmt8 expression, a region that also coincides with 5hmC enrichment. To functionally test the roles of these enzymes during eye development, we generated loss-of-function mutations in all six dnmt3-family genes, and in the tet2 and tet3 genes via TALENs. Homozygous mutants deficient in single dnmt3-family genes showed no developmental defects, most likely due to functional redundancy with other dnmt3-family genes expressed in the same regions of the embryo. To address this, we have generated double mutants deficient in dnmt3-family genes that show similar or identical expression patterns. The results of this work will elucidate requirements for epigenetic modifications governing gene expression changes during eye development.

549. Transcriptional Profiles of Ciliary Photoreceptor Neurons in Zebrafish. ***T. Shiraki***[1], *D. Kojima*[1,2], *Y. Ogawa*[1], *Y. Fukada*[1]. 1) University of Tokyo, Tokyo, Japan; 2) JST, PRESTO.
Most vertebrates have two types of morphologically distinct visual photoreceptors, rods and cones, in the retina. In addition, lower vertebrates have another type of ciliary photoreceptor neurons in the pineal gland. Rods are associated with twilight vision, whereas cones function in bright light. In contrast, the pineal photoreceptor cells receive light for non-visual purposes such as regulation of circadian rhythms. To understand molecular mechanisms underlying these physiological roles of the photoreceptor neurons, we analyzed gene expression profiles of rods, cones, and pineal photoreceptor cells. We previously found that the zebrafish has two closely related *rhodopsin* genes, *rhodopsin (rho)* and *exo-rhodopsin (exorh)*, which are specifically expressed in rods and pineal photoreceptor cells, respectively (Mano *et al.*, Brain Res., 1999). By using promoters of these genes, we established two transgenic lines, Tg(*rho:EGFP*) and Tg(*exorh:EGFP*), which express EGFP specifically in rods and pineal photoreceptor cells, respectively (Asaoka *et al.*, PNAS, 2002). Recently, Kennedy *et al.* (2006) reported the other transgenic line, Tg(*gnat2:EGFP*), expressing EGFP only in the cones and pineal photoreceptor cells. Taking advantage of these genetic resources, we isolated EGFP-positive rods, cones, and pineal photoreceptor cells by fluorescence-activated cell sorting. We then analyzed gene expression profiles of these purified photoreceptor cells by a microarray analysis and identified differentially expressed genes among photoreceptor cell types. Our microarray analysis confirmed the rod- and cone-specific expression of *phosducin* genes, *pdca* and *pdcb*, respectively, which are generated through the teleost-specific whole-genome duplication (Kobayashi *et al.*, CBP, 2002). In addition, we found three pairs of diversified teleost-specific duplicated genes that show expression patterns quite similar to the pair of *pdc* genes. It is most likely that the subtype-specific expression of these genes has been established by substantial changes in *cis*-acting elements after the global gene duplication. Further studies on promoters of these genes will reveal the *cis*-regulatory code for the subtype-specific gene expression.

550. Understanding how the oscillatory dynamics of gene expression control neurogenesis in Zebrafish. ***X. Soto***, *J. Lee*, *S.P. Herbert*, *N. Papalopulu.* Faculty of Life Science-Michael Smith Building, University of Manchester, Manchester, United Kingdom.
The dynamics of gene regulation and in particular the discovery of short period (ultradian) oscillations in gene expression, has revolutionised our ideas of the mechanisms by which neural progenitors are maintained and may underlie stem cell heterogeneity. Previous work has shown that a microRNA, miR-9, is essential for neurogenesis (1). We have shown that miR-9 controls the ultradian oscillations of Hes1, which are only sustainable within a range of concentrations of miR-9 (2). Furthermore, we have shown that pre-miR-9 is transcriptionally repressed by Hes1, and thus also likely to oscillate in progenitors. However, mature miR-9 builds over time, eventually terminating Hes1 oscillations. Therefore, we have proposed that a miR-9/Hes1 regulatory loop controls oscillations in neural progenitors and can form a tunable timer for cell differentiation. To understand the mechanism and significance of such oscillations we aim to study the dynamic regulation of gene expression in vivo using zebrafish as a system model. Zebrafish has 7 pre-miR-9 genes and we have undertaken a systematic characterization of their expression, in order to identify the best candidates for cross-regulation with Her6 (a Hes1 ortholog). We are also developing a GFP reporter line to image ultradian oscillations of her6 in vivo. Preliminary data have revealed an oscillatory behavior of the reporter in transiently expressing fish and this was abolished when the miR-9 binding site on the her6 reporter was deleted. These tools will allow us to test the hypothesis that increased miR-9 terminates oscillations, associated with neuronal differentiation or quiescence, depending on the containing final levels of Her6. (1) Coolen, M., et al., miR-9 controls the timing of neurogenesis through the direct inhibition of antagonistic factors. Dev Cell, 2012. 22(5): p. 1052-64. (2) Bonev, B., P. Stanley, and N. Papalopulu, MicroRNA-9 Modulates Hes1 ultradian oscillations by forming a double-negative feedback loop. Cell Rep, 2012. 2(1): p. 10-8.

551. Control of axon guidance of cerebellar granule cells by the basement membrane. ***M. Takeuchi***[1], *T. Hayashi*[2], *S. Yamaguchi*[2], *S. Yonemura*[3], *K. Asakawa*[4], *K. Kawakami*[4], *S. Takada*[5], *S. Kuraku*[2], *T. Shimizu*[1,2], *M. Hibi*[1,2]. 1) Bioscience and Biotechnology Center, Nagoya University; 2) Graduate School of Science, Nagoya University; 3) RIKEN Center for Developmental Biology; 4) National Institute of Genetics; 5) Okazaki Institute for Integrative Bioscience.
The cerebellum forms in the dorsal part of the most anterior hindbrain and functions in motor learning. Granule cells are the major glutamatergic neurons in the cerebellum. Granule cells in the anterior lobes of the cerebellum (valvula and corpus cerebelli) send their axons to the dendrites of Purkinje cells, whereas granule cells in the vestibule-lateral lobes (eminentia granularis and lobus caudalis cerebelli) send them to the dendrites of crest cells, Purkinje-like cells, in the dorsal hindbrain. To reveal the gene cascades that control the development of cerebellar neurons and neural circuits, we isolated zebrafish mutants for defects in the development of Purkinje and granule cells and in the formation of their neurites. *shiomaneki* mutant larvae showed shortened and/or mistargeted axons of the cerebellar granule cells in the caudal lobe. Positional cloning revealed that the *shiomaneki* gene encodes Col4a6, which is a subunit of type IV collagen and serves as a component of the basement membrane surrounding the central nervous system. Both Col4a5 and Col4a6 mutants showed the same abnormal axogenesis of cerebellar granule cells and retinal ganglion cells, indicating that the complex of Col4a5 and Col4a6 controls axogenesis of these neurons. The basement membrane structure was disorganized in Col4a5 and Col4a6 mutants. Furthermore we found similar abnormal axon targeting of granule cells in the mutants of Fibronectin1a and Integrin a5, implying that Fibronectin-Integrin signaling is required for integrity of the basement membrane containing the Col4a5/6 complex, which is subsequently utilized by the granule cells for their axogenesis. Our data suggest that the interaction of the granule cell axons with the basement membrane plays an important role in axogenesis of the granule cells. We shall also report our progress toward identification of guidance system(s) that control axon pathfinding of the granule cells by RNA sequencing.

552. Insight into the biological role of the chromatin remodeling factor CHD8 in developing zebrafish embryos. ***Jessica A. Tracy***, *Gary Kunkel*. Biochemistry and Biophysics, Texas A&M University, College Station, TX.
Accessibility of the various components of the transcription machinery to regulatory elements upstream of genes is reliant on the location and stability of nucleosomes within the region of DNA. Remodeling these regions to allow binding of transcriptional regulators depends on

the ATP-dependent movement of nucleosomes within the chromatin structure. Our focus is on the ATP-dependent chromodomain helicase DNA-binding protein 8 (CHD8). CHD8 has been shown to interact with b-catenin, p53, histone H1, and ZNF143, all factors important in regulation of transcription and modulation of expression of genes implicated in crucial developmental processes. Large-scale sequencing studies have identified mutations within CHD8 in multiple patients with autistic spectrum disorder (ASD). ASD is characterized by social impairment, repetitive and stereotyped behaviors and subtle physical cranial changes. The number of novel CHD8 mutations observed suggests that it is highly unlikely they occur by chance, which implies that mutations in one copy of CHD8 could be a main contribution to increased risk of ASD. We hypothesize that CHD8 regulates brain development in zebrafish by controlling specific genes. Whole mount in situ hybridization experiments performed in our lab have demonstrated that CHD8 is expressed in the brain and eyes during the development of these structures in zebrafish embryos. We have also observed disruption of the midbrain-hindbrain boundary after knockdown of CHD8 expression by morpholino injections. Other phenotypes observed are a change in eye size, a change in head size, and changes in the stimuli response swimming patterns of newly hatched zebrafish. These observations support our hypothesis of potential CHD8 involvement in brain development and prompt us to investigate further by generating a CHD8-knockout zebrafish. Furthermore, we are undertaking a detailed analysis of known brain markers following CHD8-knockdown in developing embryos.

553. Planar cell polarity genes control anterior-posterior guidance of Commissural Primary Ascending (CoPA) neurons in the spinal cord. *Simon D Sun, Jane K Rebman,* ***Gregory S Walsh***. Biology, Virginia Commonwealth University, Richmond, VA.
During development axonal connections are formed in the developing nervous system along the anterior-posterior (A-P) and dorsal-ventral (D-V) axis. Precise temporal and spatial gradients are responsible for guiding axons to their appropriate targets, but the precise nature of these A-P guidance cues is not yet clear in vivo. Spinal commissural neurons in the dorsal spinal cord make a series of guidance decisions en route to brain. In zebrafish, spinal commissures are initially formed by a pioneer neuron called CoPA (Commissural Primary Ascending). Like commissural neurons in other vertebrates, CoPA neurons are guided ventrally to the midline by Netrin-DCC signaling, cross the midline and are guided dorsally on the contralateral side by Slit-mediated repulsion. After midline crossing, axons of CoPA neurons turn anteriorly (rostrally) and ascend to the brain. Here, we provide evidence that anterior guidance decisions made by CoPA axons is controlled by the planar cell polarity (PCP) signaling pathway. We show using loss-of-function mutants for frizzled3a (fzd3a), van-gogh like 2 (vangl2), and scribble (scrib) that these genes are required for the anterior turning of these axons after midline crossing. Importantly, ventral and dorsal guidance of CoPA axons remains unaltered in fzd3a, vangl2, scrib mutants, suggesting that the PCP signaling pathway only controls A-P guidance decisions of CoPA axons. We also find that the majority of CoPA neurons exhibit a bias towards anterior guidance even in pre-midline crossing fibers. However, we show that a greater proportion of pre-crossing fibers grow posteriorly in PCP mutants, consistent with a role for PCP signaling controlling anterior guidance decisions of CoPA axons prior to midline crossing. Our results demonstrate that planar cell polarity signaling is required for anterior guidance decisions by pioneer CoPA axons prior to and following midline crossing.

554. Mechanism of cell loss in the hypothalamus of zebrafish *lef1* mutants. ***Yuanyuan Xie****, Richard Dorsky.* Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT.
Adult hypothalamic neurogenesis is potentially important in regulating multiple autonomic and endocrine pathways, as already shown with feeding behavior in mice (Kokoeva et al., 2005, *Science*; Sousa-Ferreira et al., 2013, *Trends Endocrinol. Metab.*). Our lab has previously shown that Wnt signaling regulates postembryonic hypothalamic progenitor differentiation in zebrafish (Wang et al., 2012, *Dev. Cell*). Consistent with our model, we showed that in mutants lacking the Wnt transcriptional mediator Lef1, the size of post-embryonic hypothalamus is reduced, due to failure of neural progenitors to differentiate (Wang et al., 2012, *Dev. Cell*). However, the specific cellular and molecular functions of Lef1 remain unknown. We find that Lef1-dependent serotonergic and other neurons begin to be lost in the hypothalamic posterior recess at as early as 3dpf, and throughout post-embryonic development. Surprisingly, the overall proliferation index does not seem to be reduced in *lef1* mutants. Further analysis suggests that another sub-population of neural progenitors prematurely differentiates into post-mitotic neurons in *lef1* mutants. BrdU pulse-chase analysis suggests that Lef1-dependent Wnt-responsive cells are primarily non-mitotic progenitors that have undergone multiple divisions before activating Wnt signaling. Therefore, we hypothesize that Lef1 directly regulates the differentiation of a quiescent progenitor pool that is specified and expanded early in development. To test this hypothesis, we are generating Cre-based lineage tracing tools to test whether Lef1-dependent Wnt-responsive cells are multipotent and self-renewing. To identify functional targets of Lef1, we are performing an RNA-seq analysis to compare the hypothalamic transcriptome from wildtype fish with that from *lef1* mutant. Together, these studies will allow us to define the specific role of Wnt signaling in hypothalamic neural progenitor maintenance and differentiation.

555. Sel1l Is Required For Zebrafish Midbrain-Hindbrain Boundary Formation. *Y. Hu, Y. Xu,* ***J. Yao***. School of Life Sciences,Fudan University, Shanghai.
Objectives: Sel1l (Suppressor enhancer lin12/Notch 1 like) encodes a protein with complex domain structure which is involved in various cellular processes including pancreatic epithelial cell differentiation, neural stem cell renewal, and cell cycle progression. Besides, sel1l is indispensable for organismal survival. However, its developmental functions are largely unknown. **Methods:** Whole mount in situ hybridization, morpholino knock-down, and mutation with TALEN and CRISP/Cas9 technology are used to investigate the roles of sel1l gene in zebrafish MHB formation. **Results:** We cloned the homolog of human sel1l, characterized its expression pattern, and investigated its developmental functions in zebrafish. Whole mount in situ hybridazation showed that sel1l expressed universally during early stage of zebrafish embryo development, and concentrated mainly in the brain after 13 somite stage. The blockage of zebrafish sel1l protein by morpholinos resulted in significantly reduction of both the midbrain and hindbrain during early embryogenesis, and the structure of the MHB isthmus was disrupted severely in the morphants. Moreover, the MHB marker gene wnt1 signal decreased during MHB induction stage while the expression domain extended along the anterior-posterior axis duirng MHB maintaince. To further explore the function and

mechanisms of sel1l during zebrafish MHB development, the sel1l deficiency mutants will be used. Now, we are generating the sel1l mutant with both the TALEN and CRISP/Cas9 technologys, and so far the F1 generation mutants have been gotton in our lab. **Conclusion:** The results implied that sel1l is required for zebrafish MHB formation and may through disturbance of wnt signaling pathway during MHB mataince. Further study will be done with the zebrafish sel1l deficiency mutants.

556. Molecular cloning, characterization and isolation of a spliced variant of zebrafish mind bomb, mib-SF. *Jae Ho Ryu[1], Jung-Woo Gwak[1], Hwan-Ki Kim[1], Dong-Won Lee[1], Ajay Chitnis[2],* ***Sang-Yeob Yeo***[1]. 1) Hanbat National University, Daejeon, South Korea; 2) 2Section on Neural Developmental Dynamics, NICHD, NIH, Bethesda, MD.
We have previously reported the characterization of a novel E3 ubiquitin ligase, Mind bomb (Mib), which promotes Notch signaling pathway by binding its ligand, Delta. To understand its functions in the initiation and progression of lateral inhibition mechanism, we now report the cloning and characterization of the splicing variant of zebrafish mind bomb (mib-SF). Mib is mapped to chromosome 2 and contains 21 exons. Northern blot detected more than two mib transcripts of approximately 2 and 4 kb. By plaque hybridization, mib-SF was isolated from oligo dT-priming cDNA library, lamda gT10. Sequence analysis and RT-PCR demonstrated that mib undergoes a alternative splicing events between exons 11 and 21 that yielded a novel splice variant, mib-SF. Putative Mib-SF protein deleted a RING finger motif which is a core domain of E3 ubiquitin ligase. Mib interacts with the intracellular domain of Jagged to promote its ubiquitylation and degradation. Our finding suggests that both Mib and Mib-SF function might be necessary for the signaling cell for efficient activation of Notch.

557. The Lowe Syndrome protein Ocrl1 is essential for zebrafish kidney function: an endocytic business. ***F. Oltrabella***[1]*, G. Piętka*[1,2]*, M. Lowe*[1]. 1) Faculty of Life Sciences, University of Manchester, Manchester, UK; 2) Research Department of Haematology, Cancer Institute, Faculty of Medical Sciences, UCL, London, UK.
Oculocerebrorenal syndrome of Lowe is a rare X-linked disorder caused by mutation of the inositol 5-phosphatase Ocrl1. Lowe Syndrome manifests as renal tubular dysfunction, neurological and ocular defects. It has been proposed that defective endocytic trafficking may be responsible for the renal tubulopathy seen in Lowe Syndrome patients, characterized by low molecular weight proteinuria and aminoaciduria, but this hypothesis is yet to be tested. Our data show that depletion of Ocrl1 can indeed cause defects in endocytosis in the zebrafish pronephric tubule. This coincides with a reduction in levels of the multi-ligand receptor Megalin, and in the abundance of the endocytic apparatus. We also show that knocking-down Pip5K in the Ocrl1 mutants can balance PtdIns(4,5)P2 level and rescue the endocytic defect. This indicates that tight control of PtdIns(4,5)P2 level is essential for efficient endocytic trafficking. Importantly, this finding suggests that Pip5K may be a valuable therapeutic target for patients with Lowe Syndrome. To further characterize the molecular mechanisms by which Ocrl1 promotes endocytosis, we have focused on the recently identified Ocrl1 interaction partners Ipip27A and B. Despite in vitro characterization, little is known about the in vivo role of Ipip27A and B. We have therefore generated Ipip27A and B mutant lines using TALEN technology. Here I will present the preliminary characterization of these mutants.

558. Sirtuins in zebrafish larvae: cooper-induced inflammation model shows altered behavior and changes in sirtuins mRNA expression. ***T.C.B. Pereira***[1]*, C.E. Leite*[2]*, L.R. Nery*[3]*, G.U. Ávila*[1]*, M.M. Campos*[2]*, M.R. Bogo*[1]. 1) Laboratório de Biologia Genômica e Molecular, PUCRS - Brazil; 2) Instituto de Toxicologia e Farmacologia, PUCRS - Brazil; 3) Laboratório de Biologia do Desenvolvimento do Sistema Nervoso, PUCRS - Brazil.
Sirtuins comprises a unique class of evolutionary conserved NAD+-dependent deacetylases that play important roles in many biological processes. Under pathological conditions, sirtuins have also been associated to inflammation, with both pro- and anti-inflammatory effects, as promising therapeutic targets. Here, we aimed to evaluate the effect of copper-induced inflammation on 7dpf zebrafish larva locomotors behavior and mRNA expression levels of all sirtuin family members (*SIRT1, 2, 3, 3.2, 4, 5, 6* and *7*). The mRNA expression of *IL-1b, IL-10, TNF-a and COX-2* were also tested as inflammation markers. The larvae (n=20/group) were treated for 4h or 24h with 10mM cooper sulfate as inflammatory agent, and immediately submitted to a five-minute-recorded assay, after a minute of acclimation, as locomotors behavior test. Total RNA was isolated and after cDNA synthesis, relative mRNA expression levels were determinate by qPCR analysis (2-DDCT method), using *EF1-a* and *Rpl13-a* as reference genes. Results were statistically compared by T-test, or one-way ANOVA followed by Tukey test, considering P£0.05 as significant. Larval swimming performance showed a significant decrease on traveled distance, mean speed and number of rotations for both 4 and 24h exposures. The inflammation mediators *IL-1b, IL-10, TNF-a* related genes showed a significant activation in all treatments, as well as *COX-2* gene for the 24h treatment, when compared to the control group. *SIRTs 2, 3, 4-6* did not present altered expression, however, we found a decrease on *SIRT1* gene expression after 4h of treatment and an activation of *SIRT7* after 24h exposure. *SIRT3.2* showed increased expression on both treatments. These findings help to ratify the inflammatory model success and also corroborate with reported potential dual-role of sirtuins in inflammation-related events.

559. A novel transgenic reporter reveals new mechanisms and roles for inducible Hsp70 expression in normal development. ***Eric A Shelden***. School of Molecular Biosciences, Washington State University, Pullman, WA.
Heat shock proteins are essential players in the cellular response to stress and have been previously shown to participate in normal developmental processes. Stress inducible members of the hsp70 family of proteins are among the most widely examined stress proteins. Surprisingly, previous studies of zebrafish have revealed a limited expression of inducible Hsp70 during normal development, with endogenous mRNA and green fluorescent protein reporter expression limited to developing lens tissues. In the present study, a stable transgenic zebrafish line was created in which approximately 2 kb of the promoter region of the stress inducible hsp70 gene was used to control expression of a yellow fluorescent protein, chosen because of its enhanced fluorescence efficiency over green fluorescent protein and reduced autofluorescence background generated by its excitation spectrum. Resulting expression patterns were examined by confocal microscopy under control conditions, after heat shock, and in embryos injected with either control or heat shock factor 1 (HSF1) specific

morpholino oligonucleotides. Consistent with previous reports, high levels of fluorescent reporter expression were observed in developing lens tissues under control conditions and ubiquitously after heat shock. However, lower but significant levels of reporter expression were also observed under control conditions in developing skeletal and cardiac muscle, gill epithelial tissues and developing brain. Injection of a morpholino inhibiting HSF1 expression, but not a control morpholino, dramatically reduced reporter expression in lens and muscle tissues, but surprisingly had a limited effect on gill and brain expression patterns. These results significantly expand the potential roles of inducible Hsp70 in normal development and suggest the presence of novel, development specific mechanisms for regulating its expression.

560. Knock-down of tapt1b causes cartilage and bone malformations in the zebrafish, resembling recessive osteogenesis imperfecta caused by TAPT1 mutations. ***Andy Willaert****, Charlotte Gistelinck, Sofie Symoens, Fransiska Malfait, Kris Vleminckx, Anne De Paepe, Paul Coucke.* Center of Medical Genetics, Ghent University, Ghent, Belgium.
Background: We recently reported a severe recessive form of osteogenesis imperfecta, caused by TAPT1 mutations, in a family with three fetuses affected with fractures of ribs and long bones, undermineralized skull and axial skeleton. TAPT1 encodes the 'Transmembrane Anterior Posterior Transformation-1' protein and is evolutionarily conserved from yeast to vertebrates. Mice that are functionally null for TAPT1 show embryonic lethality, with posterior to anterior transformations of thoracic and lumbar vertebrae. The mechanism by which this ubiquitously expressed protein causes a specific patterning defect is unknown. Therefore, using a tapt1b morphant zebrafish model we aim to identify the pathogenetic mechanisms underlying this disorder. Methods and Results: Tapt1b knock-down in zebrafish was obtained by injecting splice-blocking morpholinos (MO) in 1-4 cell stage embryos. At 4 days post fertilization (dpf) morphants presented with pericardial edema, decreased mobility, mislocalization of neural crest-derived melanocytes, microcephaly and general craniofacial malformations. Evaluation of the chondrocytes, using MO injections in fli:egfp embryos demonstrated a decrease in number and distorted deposition of the chondrocytes in the pharyngeal arch cartilage. Alcian blue staining for cartilage at 4 dpf revealed a complete absence of the pharyngeal arches and an abnormal polarity of the ceratohyal cartilage, that points to the opposite direction compared to uninjected embryos. Further, malformation and delay in ossification of the early head skeleton was observed by means of in vivo bone staining using Alizarin Complexone. Examination of morphants by acetylated a-tubulin immunostaining revealed a decrease in the number and length of the cilia in the fin buds at 24hpf, pointing to a ciliary function for tapt1b. Conclusion: We generated a morphant zebrafish model for a recessive form of osteogenesis Imperfecta caused by TAPT1 mutations. This model shows craniofacial malformations including deformation of cartilage and delayed ossification of the head skeleton and defects in cilia formation.

561. Nephron-specific Kidney Injury Molecure-1 Overexpression Induces Tubular Damage and Kidney Failure in Transgenic Zebrafish. ***W. Yin****, S. Movahedi Naini, D. Hentschel, J. Bonventre.* Renal division, BWH Boston, MA.
Background: Mammalian Kidney injury molecule-1 (KIM-1) is upregulated after kidney injury in proximal tubular cells, and serves as a highly sensitive and specific biomarker for acute kidney injury. KIM-1 is also upregulated in many subjects with chronic kidney disease. KIM-1 functions as a phagocytic receptor which mediates the uptake of apoptotic bodies as well as oxidized lipids. Data from our laboratory suggests that prolonged expression of KIM-1 in mice is maladaptive. Here, we characterized zebrafish KIM-1 (zKIM-1) family members, and studied the effect of zKIM-1 overexpression on the zebrafish pronephros. Methods: We cloned zKIM-1 and related family members and compared biochemical and functional aspects with that of human KIM-1 (hKIM-1) using PCR, western blotting, in situ hybridization (ISH) and immunostaining. We created two zebrafish models of KIM-1 overexpression in the pronephros employing the cdh17 promoter. In one case the expression was constitutive. In the other case a cdh17:CreERT2 transgenic fish line was created that allowed for tamoxifen-induced overexpression of KIM-1 in nephrons initiated at 72 hr post fertilization (hpf). Results: As with hKIM-1, zKIM-1 was not expressed in healthy nephrons, and was markedly upregulated after gentamicin-induced injury. zKIM-1 also showed a conserved phagocytic activity comparable to hKIM-1. Tissue-specific constitutive overexpression of zKIM-1 in the pronephros under cdh17 promoter control caused pericardial edema, reduced GFR and a higher mortality with defects apparent by 48 hpf. Histologic examination revealed kidney tubular damage in nephrons overexpressing zKIM-1. Cre-mediated overexpression of zKIM-1 initiated at 72hpf caused a similar kidney maladaptive phenotype marked by tubular damage, edema and higher mortality. Conclusions: zKIM-1 has high structural and functional similarities to hKIM-1. KIM-1 overexpression in the pronephros of the zebrafish results in tubular damage and kidney failure. Enhanced prolonged expression of KIM-1 in human proximal tubules may have maladaptive consequences due to KIM-1-mediated phagocytosis of tubular components.

562. Osteoclast differentiation in a medaka osteoporosis model. ***T. Yu****, A. Buettner, S. Sundaram, M. Dasyani, C. Winkler*. Department of Biological Sciences and Centre for Bioimaging Sciences, National University of Singapore, 117543 Singapore.
Mineralized bone is constantly remodelled to retain its physical properties. This requires a tightly controlled balance of bone production by osteoblasts and bone resorption by osteoclasts. In diseases such as osteoporosis, increased osteoclast and/or reduced osteoblast activity lead to reduced bone mineral density. Zebrafish and medaka have become attractive models to study bone homeostasis and model human bone diseases. Our lab established transgenic medaka to monitor osteoblast and osteoclast interactions in vivo by live imaging. We showed that overexpression of Receptor Activator of Nuclear Factor kB ligand (RANKL) leads to ectopic formation and activation of osteoclasts, evident by enhanced cathepsinK:GFP reporter expression. This resulted in increased bone resorption and a dramatic reduction of mineralized matrix. In vertebral bodies, neural and haemal arches were severely damaged or completely absent. In an attempt to rescue these defects, RANKL induced larvae were treated with two bisphosphonates, which are routinely used in human osteoporosis therapy. This resulted in an efficient block of osteoclast activity and significantly improved bone integrity in the medaka larvae, opening the possibility to use this assay for osteoporosis drug screening. RANKL induced osteoclast formation also resulted in a depletion of osteoclast progenitor pools. This coincided with up-regulation of Pu.1/spi1 and cxcr3a, two markers for the myeloid and monocytic lineages, respectively, suggesting that these cell types might be involved in osteoclast differentiation in medaka. Ectopically induced osteoclasts also affected osteoblast behaviour. Osteoblast specific osterix:mCherry expression was reduced and osteoblasts were mislocalized in the

vertebrae after RANKL induction. Furthermore, conditional ablation of osteoblasts resulted in a delay of osteoclast recruitment to regeneration sites. Together, this shows a tight interaction between osteoclasts and osteoblasts during bone remodelling, which can be visualized by live imaging in the nearly transparent medaka larvae. This work is supported by BMRC grants (07/1/21/19/544; 10/1/21/19/661) from A-STAR Singapore.

563. Regeneration of the adult zebrafish jawbone by cells with dual chondrocyte and osteoblast properties. ***Sandeep Paul***, *Simone Schindler, Alexandra De Millo Terrazzani, Francesca Mariani, Gage Crump.* Eli and Edythe Broad Institute for Regenerative Medicine and Stem Cell Research, Keck School of Medicine, University of Southern California, Los Angeles, CA.
While healing of critical size bone defects remains a major clinical challenge, some amphibians and fish robustly regenerate their skeletons. Bone repair often involves a cartilage intermediate, yet the role of chondrocytes in this process has remained unclear. Here, we describe that regeneration of the adult zebrafish lower jawbone depends on an unusual skeletal cell type with properties of both chondrocytes and osteoblasts. During jawbone regeneration, spindle shaped mesenchymal cells, marked by col1a1a expression, initially emanate out of the injured periosteum, multiply, and subsequently differentiate into col2a1a+ve cells with chondrocyte morphology. They then go on to secrete cartilage matrix while expressing genes normally associated with both chondrocytes and osteoblasts during development. Shortly thereafter, regenerating chondrocytes produce mineralized matrix and mature into osteocalcin (bglap) expressing osteocytes. In preliminary experiments, we find that the loss of regenerating chondrocytes in adult viable ihha mutants correlates with a compromised ability to repair the lower jawbone. This suggests an essential function of the cartilage callus in bone regeneration, which we are currently testing with genetic ablation strategies. We find that the regeneration of the zebrafish lower jaw bone follows a cell differentiation program that is quite different from development. Juvenile zebrafish (2-4 week post fertilization), however, show little or no overlap between osteoblast and chondrocyte markers. This is in accordance with the requirements of regeneration: restoring functionality to a damaged tissue quickly, as opposed to development, where an organism has to grow and pattern the structure over several months. Moreover, chondrocytes with osteoblast properties may also be present during mammalian bone repair, as we have observed Col10a1+; Col1a1+ chondrocytes during rib bone regeneration in mice. This rapid and large-scale regeneration of bone thus employs a conserved differentiation program that is quite different from that seen during endochondral bone development.

564. Development of Robust Adult Zebrafish Transplantation Assays Using Inbred India Mishima Strain. ***Sara Payne***, *Teresa Bowman.* Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY.
Hematopoietic cell transplantation measures hematopoietic stem cell function of donor cells upon transfer into pre-conditioned recipients. Immune matching is critical to prevent graft rejection or graft versus host reactions. Commonly used wild-type zebrafish are poorly immune matched due to genetic heterogeneity. By utilizing isogenic strains, genetic variability and other confounding factors can be reduced. In this transplant study, we compared two fish strains: AB wild type (AB) and India Mishima (IM), a recently described nearly isogenic strain. Cesium irradiation was used to condition the fish prior to transplantations. Irradiation doses sufficient for transplantation were determined for each strain of fish. For AB fish, 27.5 gy given as a split dose of irradiation resulted in 70% mortality at 30 days post irradiation (dpi) in the absence of transplantation. In contrast, IM fish treated with a split dose of irradiation totaling 30 gy showed only 20% mortality at 30 dpi, indicating a much greater radiation resistance. Thus, for transplantations, AB fish received 27.5 gy and IM fish received 32 gy. Both sets of fish were transplanted with whole kidney marrow cells isolated from ubi:GFP transgenic zebrafish. As these transgenics are closely related to the AB strain, when we transplanted into AB fish we observed engraftment in 5 out of 7 recipients, all of which showed multi-lineage chimerism. However, for IM fish, 0 out of 11 fish were engrafted likely due to the difference in immune matching between the IM and ubi:GFP strains. Generation of fluorescent donor lines in the IM nearly-isogenic background will likely be necessary to study hematopoietic stem cell function in these fish. Transplantations in the IM strain provide a new tool to understand immune system functions in zebrafish.

565. Quantitative Proteomics Screen Identifies *aldh1l1* as a Potential Regulator of Positional Memory. ***Jeremy S. Rabinowitz***[1,2,3], *Aaron M. Robitaille*[2,3], *Randall T. Moon*[1,2,3]. 1) HHMI, Seattle, WA; 2) Department of Pharmacology, University of Washington, Seattle, WA; 3) ISCRM, UW Medicine Research, Seattle, WA.
The zebrafish caudal fin has the remarkable ability to regenerate following amputation. Interestingly, amputations of varying length all complete regeneration at the same time. This ability relies on the remaining wounded tissue recalling its precise location relative to the original fin and adjusting regenerative outgrowth accordingly, an attribute known as positional memory. To identify potential novel regulators of positional memory in the caudal fin, we performed label-free protein quantification using nano liquid chromatography - tandem mass spectrometry (LC-MS/MS) on protein isolated from dorsal-proximal and ventral-distal regions of 20 AB WT fish (1:1 gender ratio). We quantified 1708 individual proteins, with only 16 genes showing at least 4-fold expression level differences in proximal vs. distal regions. *Aldehyde dehydrogenase 1 family, member L1* (*aldh1l1*), an enzyme involved in folate metabolism, is one hit that showed increased proximal expression. Alterations in expression have been validated at the transcript level using qRT-PCR and at the protein level by Western blot. If *aldh1l1* is involved in positional memory, knocking protein levels down in a proximal amputation (to mimic distal levels) should result in decreased regenerative proliferation and decreased outgrowth. To test this, we injected antisense morpholino oligonucleotides targeting *aldh1l1* into the dorsal half of a proximally amputated fin and found ~50% inhibition in regenerative growth, as compared to the uninjected ventral side. These findings suggest *aldh1l1* may help regulate positional memory in the caudal fin. Furthermore, our screen has identified several additional proteins differentially expressed in proximal and distal regions of the fin, which are also strong candidates for genes regulating positional memory.

566. Notch Signaling Maintains Stem Cell Compartments by Controlling Cell Division Patterns During Hair Cell Regeneration in the Zebrafish Lateral Line. ***Andres Romero-Carvajal***[1,2], *Linjia Jiang*[1], *Agne Kozlovskaja-Gumbriene*[1], *Tatjana Piotrowski*[1]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Neurobiology and Anatomy. The University of Utah. Salt Lake City, UT.
Sensory hair cell damage leads to permanent hearing loss and is the most common sensory disability. The zebrafish mechanosensory organs (neuromasts) are an excellent model for the study of hair cell regeneration. Neuromast have a central group of sensory hair cells surrounded by two populations of support cells, the inner support cells and the mantle cells. While the adult mammalian inner ear hair cells can't be regenerated, neuromasts have the ability to do so via inner support cell proliferation. It is not known whether the inner support cells or the mantle cells are stem cells and how heterogeneous these two cell populations are. To address these questions, we used confocal live imaging and BrdU analyses of regenerating and homeostatic neuromasts. Data from 72-hour recordings show that inner support cells are highly proliferative after hair cell death, whereas mantle cells do not respond. Inner support cells either self renew (amplifying division) or give rise to a pair of hair cells (differentiating division). During homeostasis and regeneration, amplifying divisions occur in discrete dorsal and ventral regions of the sensory organ, whereas differentiating divisions occur centrally in close proximity to hair cells. It has been previously suggested that the use of g-secretase inhibitors enhances hair cell regeneration by increasing proliferation. Here we demonstrate that loss of Notch signaling does not affect proliferation, but that it causes a shift from amplifying to differentiating cell divisions leading to the loss of self-renewing progenitors. Thus Notch signaling is required to maintain pools of amplifying support cells in the poles in cross talk with multiple signaling pathways that together maintain tissue homeostasis by balancing proliferation and differentiation.

567. ZF143 Enhances Heart and Complex Appendage Tissue Regeneration in Zebrafish. ***Ashley M Smith***[1], *Helen Roberts*[1,2], *Heather R Carlisle*[1], *Viravuth P Yin*[1]. 1) Davis Center for Regenerative Biology and Medicine, Mount Desert Island Biological Laboratory, Salisbury Cove, ME; 2) Tufts University, Medford, MA.
The absence of a robust regenerative capacity underscores numerous chronic and degenerative diseases in humans. For instance, adult mammals will repair injured hearts through the formation of non-contractile collagen laden scar tissue. In contrast, the adult zebrafish is endowed with the remarkable capacity to regenerate all damaged heart tissue to fully restore heart function throughout its life. Research in our lab and others have shown that the mechanism of cardiac tissue regeneration is conserved from fish to mice to humans. For reasons that remain unknown, these regenerative circuits are muted within a week after birth in mammals. In this study, we used the adult zebrafish to screen a limited number of natural compounds in order to identify molecules that may reawaken genetic networks that underscore regenerative capacity. We identified ZF143, a naturally occurring compound that greatly enhances regeneration of heart and appendage tissues. ZF143 increases cardiomyocyte proliferation indices by over 200% when compared with control animals. This enhancement in proliferation is accompanied with decreases in scar tissue deposition within the wounded apex. Importantly, ZF143 is able to restore regenerative capacity in zebrafish models with repressed heart regenerative capacity. ZF143 can also significantly increase the rate of appendage regeneration by as much as 200% and without promoting tissue overgrowth. During appendage regeneration, ZF143 enhances cellular dedifferentiation and proliferation of progenitor cells. Collectively, this data suggests ZF143 could have strong therapeutic applications for increasing the capacity of regeneration in both the heart and limb in mammals.

568. Progenitor cell maintenance in the postembryonic retina: the role of a novel regulator of small nuclear RNA expression. ***Natalie Sorfazlian***[1], *Monica Dixon-Fox*[1], *Jason Willer*[2], *Ronald Gregg*[2], *Vincent Tropepe*[1]. 1) Department of Cell and Systems Biology, University of Toronto, Toronto, Canada; 2) Department of Biochemistry & Molecular Biology, University of Louisville, USA.
Maintaining a discrete population of retinal progenitor cells in the circumferential marginal zone (CMZ) is required for the proper growth of a functional vertebrate retina after embryogenesis is completed. In the zebrafish mutant called *kes*^{s546}, the CMZ is reduced in size compared to the wild-type retina and cells no longer express markers of cycling retinal progenitors, such as *ccnd1*. Mutant CMZ cells have defects in cell polarity with an apparent loss of epithelial integrity leading to increased apoptosis. In addition, there is a progressive overgrowth of the retinal-pigmented epithelium (RPE) at the margin in these mutants over time. BrdU pulse-chase experiments are being conducted to determine if surviving CMZ cells are converting to an RPE identity or whether peripheral RPE cells independently expand as a consequence of the loss of the CMZ. We mapped this mutation to the *kiaa0947l* gene, which is orthologous to *Drosophila* and human *ICE1*. Pol II-dependent transcription of snRNA genes requires the little elongation complex, or LEC, which is composed of ELL, ICE1, ICE2, EAF, and USPL1 proteins. The assembly of this complex at snRNA gene loci requires ICE1 to recruit Pol II during transcription initiation. We are currently investigating the underlying causes of these progenitor cell defects, including the effect on transcription of Pol II-dependent snRNAs in kes mutants. Our analysis of *kes* provides critical insight into the role of LEC dependent small RNA expression in regulating progenitor cell maintenance in the postembryonic retina.

569. Cocaine alters the daily patterns of adult neurogenesis. ***Alexander Stankiewicz***[1], *Veronica Akle*[2], *Konstantin Kopotiyenko*[1], *Lili Yu*[1], *Sharon Fan*[1], *Christina Teng*[1], *Irina Zhdanova*[1]. 1) Anatomy and Neurobiology, Boston University, Boston, MA; 2) Medicine, Universidad de los Andes, Bogotá, Colombia.
Adult neurogenesis in vertebrates occurs in discrete regions of the central nervous system. The milieu specific to promoting proliferation in these neurogenic niches can vary in a circadian manner and might be fundamentally altered by pharmacologic agents, including drugs of abuse. To address this, we used the zebrafish model to determine whether adult neurogenesis undergoes circadian variation and if cocaine interferes with this process. The principal advantages of the model are abundant adult neurogenesis, strong circadian rhythms and established responsiveness to cocaine in this species. Moreover, zebrafish provides important benefits of a diurnal vertebrate model for translational circadian research into the effects of drugs of abuse in diurnal humans. Using BrdU labeling, expression levels for four cyclins and a kinase inhibitor that controls specific phases of the cell cycle, we documented a robust circadian rhythm of cell proliferation in adult brain. We then demonstrated two distinct effects of cocaine on this process. Acutely, cocaine augments the S phase of the cell cycle at the

time of the circadian peak in neurogenesis, in the late evening. In contrast, the morning administration of cocaine lacks this acute effect but leads to reduced cell proliferation the night after. The results are consistent with and will be discussed in the context of our hypothesis of the "two-night circadian regulation of adult neurogenesis".

570. Characterization of Sonic Hedgehog Function during Retinal Regeneration in Adult Zebrafish. ***Ryan Thummel***, *Jennifer Thomas.* Wayne State University School of Medicine, Detroit, MI.
In contrast to the mammalian retina, the zebrafish retina possesses the remarkable ability to regenerate following damage. It is well established that this is primarily accomplished through Müller glial cells. Upon damage, Müller glial cells re-enter the cell cycle to form progenitor cells, which then migrate to the area of damage and differentiate into new neurons. The purpose of this study is to identify the signals that affect Müller glial cell proliferation and subsequent differentiation of retinal progenitors. A recent report implicated Sonic Hedgehog (Shh) signaling in the proliferation of Müller glial-derived progenitors. In order to definitively determine the role of Shh signaling during adult retinal regeneration we used gain- and loss- of function techniques and two retinal damage models: constant intense light to specifically ablate photoreceptors, and intravitreal injections of the cytotoxin Ouabain to damage all retinal neurons. Using the light-damage model, we first show that Shh signaling induces Müller glial reactive gliosis, including cell hypertrophy and an up-regulation of glial fibrillary acidic protein. In addition, Shh regulates the percentage of Müller glial cells that re-enter the cell cycle following damage and exhibits neuroprotective effects on both rod and cone photoreceptors. Next, using the Ouabain-damage model, we show that Shh signaling also affects the differentiation of retinal progenitors. An increase in Shh signaling specifically increases the number of amacrine and ganglion cells in the regenerated retina, whereas inhibiting Shh signaling results in fewer amacrine and ganglion cells. This result is consistent with the defined role of Shh during retinal development, when Shh signaling is required for differentiation of amacrine and ganglion cells. Together, these data define the pleiotropic roles of Shh during retinal regeneration and add to the growing list of signaling pathways that regulate the regenerative response of Müller glial cells in the adult zebrafish retina.

571. Wnt/beta-catenin signaling orchestrates fin regeneration. *Gilbert Weidinger[1], Wiebke Cizelsky[1], Mohankrishna Dalvoy[1], Günes Özhan[1], Richard Dorsky[4], Enrico Moro[2], Francesco Argenton[3], Michael Kühl[1],* ***Gilbert Weidinger***[1]. 1) Inst. for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany; 2) Department of Molecular Medicine, University of Padova, Padova, Italy; 3) Department of Biomedical Sciences, University of Padova, Padova, Italy; 4) Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, USA.
Adult zebrafish regenerate the caudal fin following amputation via dedifferentiation of mature stump cells, which form a proliferative population of progenitor cells, the blastema. During regenerative growth, Wnt/beta-catenin signaling is essential for blastemal proliferation and patterning of the overlying epidermis. Surprisingly however, we find that beta-catenin signaling is not active in the epidermis nor the majority of the proliferative blastemal cells. Rather, signaling is confined to specific sub-regions of the blastema, in particular a small group of non-proliferative cells in the distal blastema, plus pre-osteoblasts and cells lining the osteoblast precursors in the proximal, proliferative blastema; the latter group of cells is thought to form non-mineralized skeletal elements, the actinotrichia. Intriguingly, tissue-specific pathway interference using the TetON system indicates that Wnt signaling is not essential in the epidermis nor in committed osteoblasts, while signaling in the non-proliferative distal blastema is required for cell proliferation in the proximal blastema, and signaling in the actinotrichia-forming cells is required for differentiation of adjacently located osteoblasts. Thus, it appears that Wnt signaling regulates epidermal patterning, blastemal proliferation and osteoblast maturation at least in part indirectly via secondary signals. Gene expression profiling, chromatin immunoprecipitation and functional rescue experiments suggest that Wnt/beta-catenin signaling acts through Fgf and Bmp signaling to control epidermal patterning, while retinoic acid and Hedgehog signals mediate its effects on blastemal cell proliferation. Together, our results suggest that the Wnt/beta-catenin pathway orchestrates fin regeneration by defining organizing centers that instruct cellular behaviors of adjacent tissues.

572. Investigating the Role of Innate Immune Cells in Retinal Regeneration. ***David T. White***[1,2], *Jeff S. Mumm*[1]. 1) Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD; 2) Neuroscience, Georgia Regents University, Augusta, GA.
Humans do not spontaneously repair retinal damage, thus we are susceptible to numerous injurious and degenerative disease conditions. Our long-term goal is to restore visual function to patients via therapeutics that stimulate dormant regenerative capacities in the human eye. Zebrafish display a robust capacity for repairing neural tissues, including the retina. We hypothesize that identifying mechanisms that regulate the regenerative potential of Müller glia—a conserved retinal stem cell—will provide insights useful in the development of regenerative therapies. Here, we have begun to explore roles played by resident macrophages/microglia and neutrophils during the selective loss and regeneration of rod photoreceptors in the zebrafish retina (a physiological model of retinitis pigmentosa). To account for the dynamic/complex nature of reactive immune cells, we leveraged an approach facilitated by the zebrafish system: in vivo time-lapse imaging. This allowed us to typify resident macrophage/microglia responsiveness to rod photoreceptor ablation, defining a temporal window of immune system activation characterized by initial migration toward and subsequent phagocytosis of dying rod cell fragments. Immunohistochemistry was used to further detail macrophage/microglia and stem cell responses to cell loss—e.g., proliferation. Additionally, we co-ablated either microglia or neutrophils with rod cells to determine whether specific immune cell types play beneficial or deleterious roles during retinal regeneration. These studies revealed that the rate of debris clearance and kinetics of regeneration were dependent on the presence of select immune cell types. This work has begun to define how the innate immune system interacts with Müller glia to regulate regenerative responses to selective cell loss in the retina. Determining how innate immune cells shape regenerative processes will help to inform novel therapeutic strategies—e.g., modulating immune system responsiveness to retinal cell loss to promote stem cell proliferation—aimed at reversing vision loss caused by degenerative retinal conditions.

573. Glucagon signaling is enssential for beta cell neogenesis and transdifferentiation from alpha cells in zebrafish. ***L. Ye***[1], *M. Robertson*[1], *D. Hesselson*[2,3], *D. Stainer*[2,4], *R. Anderson*[1]. 1) Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN; 2) Department of Biochemistry and Biophysics,University of California San Francisco,CA; 3) Garvan Institute of Medical Research, Metabolic Diseases Division,Sydney, Australia; 4) Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Ludwigstr, German.
The inter-conversion of cell lineages via transdifferentiation is an adaptive mode of tissue regeneration, and an appealing therapeutic target. However, its clinical exploitation is contingent upon discovery of contextual regulators of cell fate acquisition and maintenance. In murine models of diabetes, glucagon-secreting alpha cells transdifferentiate into insulin-expressing beta cells following targeted beta cell depletion, regenerating the form and function of the pancreatic islet. However, the molecular mediators of this mode of regeneration are unknown. Here, using lineagetracing assays in a transgenic zebrafish model of beta cell ablation, we demonstrate conserved plasticity of alpha cells during islet regeneration. In addition, we show that glucagon expression is upregulated after injury, and through gene knockdown and rescue approaches, that it is necessary for alpha to beta cell fate switching. Importantly, while beta cell neogenesis was stimulated by glucose, alpha to beta cell conversion was not, suggesting that transdifferentiation is not mediated by glucagon control of hepatic glucose production. Overall, this study supports the hypothesis that alpha cells are a deep endogenous reservoir of potential new beta cells. It further reveals that glucagon plays an important role in maintaining endocrine cell homeostasis through feedback mechanisms that govern cell fate stability.

574. Hydrogen Peroxide Primes Heart Regeneration with a Derepression Mechanism. *Peidong Han*[1], ***Xiao Hai Zhou***[1], *Nannan Chang*[1], *Cheng-Lu Xiao*[1], *Shouyu Yan*[1], *He Ren*[2,4], *Xin-Zhuang Yang*[1], *Mei-Ling Zhang*[1], *Qing Wu*[1], *Boyang Tang*[1,2], *Ju-Peng Diao*[1], *Xiaojun Zhu*[1], *Chuanmao Zhang*[2,4], *Chuan-Yun Li*[1], *Heping Cheng*[1,2,3], *Jing-Wei Xiong*[1]. 1) Beijing Key Laboratory of Cardiometabolic Molecular Medi cine and Institute of Molecular Medicine; 2) State Key Laboratory of Biomembrane and Membrane Biotechnology; 3) The Peking-Tsinghua Center for Life Sciences; 4) College of Life Sciences, Peking University, Beijing 100871, China.
While the adult human heart has very limited regenerative potential, the adult zebrafish heart can fully regenerate after 20% ventricular resection. Although previous reports suggest that developmental signaling pathways such as FGF, PDGF and RA are reused in adult heart regeneration, the underlying intracellular mechanisms remain largely unknown. Here we show that H_2O_2 acts as a novel epicardial and myocardial signal to prime the heart for regeneration in adult zebrafish. Live imaging of intact hearts revealed highly localized H_2O_2 (~30 mM) in the epicardium and adjacent compact myocardium at the resection site. Decreasing H_2O_2 formation with the Duox inhibitors DPI or apocynin, or scavenging H_2O_2 by catalase overexpression markedly impaired cardiac regeneration, indicating that H_2O_2 is an essential signal in this process. Mechanistically, elevated H_2O_2 destabilized the redox-sensitive phosphatase Dusp6 and hence increased the phosphorylation of Erk1/2. The Dusp6 inhibitor BCI achieved similar pro-regenerative effects while transgenic overexpression of dusp6 impaired cardiac regeneration. H_2O_2 plays a dual role in recruiting immune cells and promoting heart regeneration through two relatively independent pathways. We conclude that H_2O_2 potentially generated from elevated Duox/Nox2 promotes heart regeneration in zebrafish by unleashing MAP kinase signaling through a derepression mechanism involving Dusp6.

575. Expressed repeat elements improve RT-qPCR normalization across a wide range of zebrafish gene expression studies. ***Suzanne MA Vanhauwaert***, *Gert Van Peer, Ali Rihani, Pieter Rondou, Steve Lefever, Anne De Paepe, Paul Coucke, Frank Speleman, Jo Vandesompele, Andy Willaert.* Center for medical genetics, Ghent University, Ghent, Belgium.
The selection and validation of stably expressed reference genes is a critical issue for proper RT-qPCR data normalization. In zebrafish expression studies, many commonly used reference genes are not generally applicable given their variability in expression levels under a variety of experimental conditions. Inappropriate use of these reference genes may lead to false interpretation of expression data and unreliable conclusions. In this study, we evaluated a novel normalization method in zebrafish using expressed repetitive elements (ERE) as reference targets, instead of specific protein coding mRNA targets. We assessed and compared the expression stability of a number of EREs to that of commonly used zebrafish reference genes in a diverse set of experimental conditions including a developmental time series, a set of different organs from adult fish and different treatments of zebrafish embryos including morpholino injections and administration of chemicals. Using geNorm and rank aggregation analysis we demonstrated that EREs have a higher overall expression stability compared to the commonly used reference genes. Moreover, we propose a limited set of ERE reference targets (loopern4, hatn10 and dna15ta1), that show stable expression throughout the wide range of experiments in this study, as strong candidates for inclusion as reference targets for qPCR normalization in future zebrafish expression studies. Our applied strategy to find and evaluate candidate expressed repeat elements for RT-qPCR data normalization has high potential to be used also for other species.

576. *dync2h1* mutants reveal a role for cilia in neomycin-induced hair cell death. ***Tamara Stawicki***[1], *Robert Esterberg*[1,2], *Tor Linbo*[1], *Kelly Owens*[1], *Edwin Rubel*[1,2], *David Raible*[1,2]. 1) University of Washington, Seattle, WA; 2) Virginia Merrill Bloedel Hearing Research Center, Seattle Washington.
Hearing loss as a result of hair cell death is a dose limiting side effect of multiple therapeutic drugs, including aminoglycoside antibiotics and chemotherapeutics. We have used the zebrafish lateral line system to screen for mutations that confer protection against hair cell death in response to neomycin. Through this screening we have identified a mutation in the *dynein cytoplasmic 2 heavy chain 1* gene (*dync2h1*). *dync2h1* has been implicated in other systems in retrograde intraflagellar transport in cilia. *dync2h1* mutants show the curved body phenotype typically seen in cilia mutants. *dync2h1* is the second cilia gene identified through this screen, the first being the transition zone gene *cc2d2a*. Hair cells of the lateral line in *dync2h1* mutants appear to develop normally, however, by 5 dpf their kinocilia are dramatically shortened and there is a reduction in total hair cell number. These phenotypes are similar to what has previously been reported in the anterograde intraflagellar transport gene *ift88* and is in contrast to *cc2d2a* mutants that show grossly normal kinocilia morphology. Stereocilia morphology and polarity on the other hand is normal in both mutants. Using fluorescently labeled neomycin we have found that

neomycin uptake into hair cells is largely unchanged in both cilia mutants suggesting these genes are playing a role in hair cell death downstream of uptake. Future experiments will look at other cilia mutants to delineate the cilia signaling pathways responsible for modulation of neomycin-induced hair cell death.

577. Ablation of the embryonic vasculature causes defects in retinal development of zebrafish embryos. *Susov Dhakal[1], **Craig Stevens**[2], Deborah Stenkamp[1]*. 1) Department of Biological Sciences, University of Idaho, Moscow, ID; 2) Biology Department, College of Wooster, Wooster, OH.
The endothelial cells of the hyaloid vasculature surrounding the lens differentiate at approximately the same time as retinal neurogenesis begins in the developing zebrafish eye. The timing suggests a potential for critical interactions between the developing eye vasculature and formation of retinal neurons. This was investigated by selectively ablating the vasculature using multiple methods. In the first, two transgenic lines: Tg(cdh5:gal4) and Tg(UAS-E1b:Eco.NfsB-mCherry) were crossed in order to express nitroreductase in endothelial cells expressing cadherin5. Embryos were treated with the drug metronidazole (mtz), which reacts with nitroreductase to generate a toxic product that kills cells expressing the enzyme. Transgenic embryos were targeted with mtz at 12 hours post-fertilization (hpf) which led to absence of retinal vasculature during the period of retinal neurogenesis. As an alternative strategy, wildtype zebrafish embryos were treated with the VEGF receptor signaling inhibitor SU5416 for 2 hours at 20 hpf which blocks formation of blood vessels in the embryo. Both mtz-treated transgenic and SU5416-treated embryo had significant defects in size of eye and lens and abnormal retinal lamination. Compared to untreated embryos, eye size was smaller (microphthalmia), with disrupted lamination between the inner and outer retinal layers. By 72 hpf there was a significant reduction in the presence of rods and red and green cone photoreceptors compared to untreated embryos. The potential role of hypoxia due to lack of blood circulation in the eye was investigated by examining silent heart mutants, which have the retinal vasculature but lack a contractile heart pushing fluids through the circulatory system. Results suggest that retinal neurogenesis in silent heart is much less affected, with no significant reduction in cones but a significant reduction in numbers of rod photoreceptors. The conclusion is that the presence of vasculature endothelial cells in the zebrafish retina is essential and has a paracrine function regulating retinal neurogenesis.

578. Otogelin and Alpha-Tectorin Are Required for Correct Otolith Tethering in the Zebrafish Inner Ear. *Georgina A. Stooke-Vaughan[1], Nikolaus D. Obholzer[2], Sarah Baxendale[1], Sean G. Megason[2], **Tanya T. Whitfield**[1]*. 1) Bateson Centre, Department of Biomedical Science, University of Sheffield, Sheffield, S10 2TN, UK; 2) Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA.
Otoliths are biomineralised structures important for balance and hearing in fish. In the adult ear, they are held in place by the otolithic membrane, an acellular matrix that provides a physical coupling between the otolith and its associated sensory epithelium. The development and composition of this membrane have not previously been described in zebrafish. Our data indicate that there are at least two stages of otolith tethering in the zebrafish, and we have identified two components of the otolithic membrane through analysis of mutant lines with defects in otolith tethering. Initiation of tethering, which involves the binding of otolith precursor particles to the tips of sensory hair cell kinocilia, requires the function of the *otogelin* gene, disrupted in the *einstein* (*eis*) mutant. Maintenance of otolith tethering during later larval stages requires the function of *tecta*, which is disrupted in the *rolling stones* (*rst*) mutant. The *tecta* gene codes for Alpha-Tectorin, a protein that localises strongly to the otolithic membrane over each sensory patch in the wild-type ear. Mutations in the human *OTOG* gene are a known cause of autosomal recessive deafness and vestibular problems; mutations in the human *TECTA* gene can cause either recessive (DFNB21) or dominant (DFNA8/12) forms of deafness. Our findings confirm the zebrafish *eis* and *rst* mutants as new models of these human disorders.

579. The olfactory sensory system develops from coordinated movements within the neural ectoderm. ***Kathleen Whitlock**, Jorge Torres-Paz.* Centro Interdisciplinario de Neurociencia, Univ. de Valparaiso, Valparaiso, Chile.
Background: The peripheral olfactory sensory system arises from morphologically identifiable structures called placodes. Placodes are a relatively late-developing structures, evident only well after the initiation of somitogenesis. Placodes are generally described as being induced from the ectoderm with the insinuation that their development is separate from the coordinated cell movements generating the central nervous system. Results: With the advent of modern techniques it is possible to follow development of the neurectoderm giving rise to the anterior neural tube, including the olfactory placodes. The cell movements giving rise to the optic cup are coordinated with those generating the olfactory placodes and adjacent telencephalon. The formation of the basal lamina separating the placode from the neural tubes is coincident with the anterior migration of cranial neural crest. Conclusions: The transient morphological structures called olfactory placodes are a product of the continuous sheet of neurectoderm specified at the end of gastrulation. The olfactory placodes and telencephalon are generated from complex cell movements within the developing neural plate similar to that observed for the developing optic cup. Research funding: NIH/NIDCD 050820 (KW); FONDECYT 1111046 (KW); Instituto Milenio ICM- P09-022.F (KW); CONICYT21110200 (JT).

580. Toward the identification of genetic modifiers of the ocular coloboma phenotype in Gorlin Syndrome. ***Emily O Wirick**, Kristen M Kwan.* Human Genetics, University of Utah, Salt Lake City, UT.
The choroid fissure is a transient, yet critical structure that develops at the ventral side of the eye. Its development creates the channel through which vasculature will enter the eye and retinal axons exit. Defects in the choroid fissure result in uveal coloboma, a human birth defect associated with visual impairment. Mutations in the human *PATCHED* gene result in Gorlin Syndrome, characterized by medulloblastoma and basal cell carcinoma due to overactive Hedgehog signaling. Dozens of other symptoms are associated with Gorlin Syndrome, including coloboma, which occurs with variable penetrance: the mechanisms causing coloboma are unknown. Like humans, mutations in zebrafish *patched2* lead to coloboma with variable penetrance. Our goal is to determine genetic factors that affect the penetrance of the coloboma phenotype in a zebrafish model of Gorlin Syndrome. It has been previously reported that mutation in zebrafish

patched2 leads to coloboma with 80% penetrance, assuming that 100% penetrance represents all homozygous mutant embryos developing the phenotype. We began with a group mating from 3 heterozygous pairs of *patched2* mutants, and have generated two generations. When F2s are isolated as single heterozygous pairs, we find three outcomes: pairs that yield 40% penetrance (low penetrance), others that yield 80% penetrance (intermediate penetrance), and still others that yield 120% penetrance (high penetrance). Penetrance values for a particular pair are highly reproducible upon remating. Genotyping confirms that 40% penetrance represents 40% of mutants with coloboma, with 60% of mutants showing no phenotype. 120% penetrance represents all homozygous mutants plus a fraction of heterozygous embryos with coloboma, suggesting that something in these pairs uncovers haploinsufficiency of *patched2*. We are performing crosses between low and high penetrance individuals to determine the nature of the genetic differences. We plan to use SNP chip analysis or whole genome sequencing to determine the genetic basis of coloboma penetrance in *patched2* mutant zebrafish. We hope to identify one or more factors that affect Hedgehog signaling and choroid fissure development, which may yield insight into the human condition.

581. The Role of Neprilysin in Diabetic Nephropathy. *R Powell[1], Y Fukuyo[1], Y Kawashima[2,3], Y Kodera[3], S Terai[4], T Sakai[4], M Elliott[5,6], H Matsumoto[2], **T Obara**[1]*. 1) Cell Biology, OUHSC, Oklahoma City, OK, USA; 2) Biochemistry and Molecular Biology, OUHSC, Oklahoma City, OK, USA; 3) Physics, Kitasato University School of Science, Sagamihara, Kanagawa, Japan; 4) Gastroenterology and Hepatology, Yamaguchi University, Graduate School of Medicine, Ube, Yamaguchi, Japan; 5) Ophthalmology, OUHSC, Oklahoma City, OK, USA; 6) Dean McGee Eye Institute, OUHSC, Oklahoma City, OK, USA.
Hyperglycemia is a manifestation of diabetes that leads to diabetic nephropathy. It progresses to end-stage renal disease requiring dialysis or kidney transplant. The Renin-Angiotensin system (RAS) plays a pathogenic role in various renal disorders. Neprilysin is a membrane metallo-endopeptidase expressed in the glomerulus and apical membrane of proximal and distal tubules. It regulates peptides modulating lipid metabolism and glucose maintenance. The roles of Neprilysin and RAS in diabetic nephropathy and hyperglycemia are unknown. We found that Neprilysin is downregulated in kidneys in several models of diabetes, including mice and medaka fed a high fat diet (HFD) and rats treated with streptozotocin. Results were confirmed by proteomics, western blot, and subcellular immunohistochemical analysis. neprilysin knockdown in mice and adult medaka resulted in diabetic nephropathy and decreased kidney function in response to hyperglycemia. Neprilysin regulates the peptides modulating lipid metabolism and glucose maintenance. Downregulation of renal Neprilysin reduced levels of angiotensin-(1-7). Treatment with telmisartan, an angiotensin II receptor blocker, recovered angiotensin-(1-7) levels, and prevented nephropathy and hyperglycemia induced by either HFD or NEPRILYSIN knockdown in medaka. These data support our hypothesis that kidney Neprilysin protein is indispensable for preventing diabetic nephropathy and modulating hyperglycemia by maintaining a balance between angiotensin-(1-7) and angiotensin II. Our combination of an innovative discovery-based approach followed by genetic validation in adult animal models will provide mechanistic insight into the roles of Neprilysin, and unveils the potential of new therapeutic approaches.

582. During melanocyte differentiation, widely-expressed transcription factor TFAP2A co-activates many of the same transcriptional targets as lineage-specific transcription factor MITF. ***Hannah Seberg**[1], Eric Van Otterloo[2], Gregory Bonde[2], Robert Cornell[1,2]*. 1) Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA.
Here we examine the respective roles of the lineage-specific microphthalmia-associated transcription factor (MITF), and the widely-expressed transcription factor activator protein 2 alpha (TFAP2A) in terminal differentiation of melanocytes. MITF expression is confined to the melanocyte lineage (at least within the neural crest), and MITF directly activates many melanocyte-specific differentiation effectors, such as genes involved in melanin synthesis. By contrast, TFAP2A is expressed in multiple differentiated cell types including keratinocytes, kidney cells, several types of neurons, and melanocytes. In zebrafish melanocytes, a second paralog, *tfap2e*, is co-expressed. To study the role of TFAP2 paralogs in melanocyte differentiation, we created a *tfap2e* mutant using zinc finger nucleases and crossed it to a *tfap2a* mutant. In *tfap2a/e* double mutants, embryonic melanocytes are reduced in number by about 50%, and their differentiation is profoundly delayed. However, expression of *mitfa* appears normal in these melanocytes. While *tfap2a* and *mitfa* single heterozygous embryos are phenotypically normal, *tfap2a;mitfa* double heterozygotes have fewer embryonic melanocytes. These data reveal that TFAP2A and MITF interact genetically, but the biochemical basis of this interaction is unclear. One possibility is that TFAP2A and MITF directly co-activate genes that promote melanocyte growth and differentiation. To test this model, we conducted anti-TFAP2A ChIP-Seq in human melanocytes. We then generated a gene expression profile from trunks of *tfap2a* null zebrafish embryos. Genes at the intersection of these profiles are likely to be directly regulated by TFAP2A. Among these are many MITF-target genes, including those encoding growth factor receptors (MC1R and EDNRB), transcription factors (SOX10), and notably, melanin-synthesis genes (DCT, PMEL, and OCA2). These results show that “generic” transcription factors like TFAP2 can activate expression of lineage-specific targets.

583. Ikk2 regulates tissue integrity and cytokinesis during zebrafish development. ***Hongyuan Shen**, Vinay Tergaonkar, Vladimir Korzh.* Institute of Molecular and Cell Biology, Singapore, Singapore, Singapore.
NFkB is a family of transcription factors which regulates of a wide range of cellular targets, including chemokines, immune receptors, adhesion molecules, stress response genes, regulators of apoptosis, transcription factors, growth factors, enzymes and cell cycle regulators. IKK1 and IKK2 are kinases essential for activation of NFkB signaling in vertebrates. To analyze the role of Ikk2 during early vertebrate development, we used the zinc-finger nuclease (ZFN) mediated mutagenesis to generate the two different alleles of Ikk2 mutant in zebrafish. Their analysis demonstrated that the zygotic function of Ikk2 is required during embryogenesis to establish vascular integrity. This deficiency is transient and despite some hemorrhage fish are able to compensate later on and some of them develop into fertile adults. Homozygous mutants are prone to skin abrasions, which either happens spontaneously or more frequently due to mechanically stressful conditions such as mating. A clue as to a mechanism involved came in result of analysis of the maternal function of Ikk2, which revealed its requirement in cell adhesion and cytokinesis.

584. Modulation of Estrogen Causes Disruption of Craniofacial Chondrogenesis in *Danio rerio*. ***Benjamin S. Walker****, Sarah P. Cohen, Adam R. LaChappelle, Christopher S. Lassiter.* Roanoke College, Salem, VA.
Estrogen is a steroid hormone that is ubiquitous in vertebrates, but its role in cartilage formation has not been extensively studied. Abnormalities of craniofacial cartilage and bone account for a large portion of birth defects in the United States. Zebrafish (*Danio rerio*) have been used as models of human disease, and their transparency in the embryonic period affords additional advantages in studying craniofacial development. In this study, zebrafish embryos were treated with 17-b estradiol (E2) or with an aromatase inhibitor and observed for defects in craniofacial cartilage. Concentrations of E2 greater than 2 mM caused major disruptions in cartilage formation. Concentrations below 2 mM caused subtle changed in cartilage morphology that were only revealed by measurement. The angles formed by cartilage elements in fish treated with 1.5 mM and 2 mM E2 were increasingly wide, while the length of the primary anterior-posterior cartilage element in these fish decreased significantly. These treatments resulted in fish with shorter, flatter faces as estrogen concentration increased. Inhibition of aromatase activity also resulted in similar craniofacial disruption indicating that careful control of estrogen signaling is required for appropriate development. Further investigation of the phenomena described in this study could lead to a better understanding of the etiology of craniofacial birth defects.

585. Characterization of the Zebrafish Ortholog of Zipper Interacting Protein Kinase (ZIPK/DAPK3). ***Douglas C. Weiser****, Brandon Carr.* Biological Sciences, University of the Pacific, Stockton, CA.
Reversible phosphorylation of the type II myosin regulatory light chain (MLC2) is a critical regulatory mechanism for controlling type II myosin and the actin cytoskeleton. Precise control of MLC2 phosphorylation is required for numerous cellular processes including morphogenetic cell movements during development, smooth muscle contraction and tumor cell invasion. MLC2 is phosphorylated, by a number of protein kinases, and dephosphorylated primarily by a highly conserved myosin phosphatase (MP) complex. MLC2 kinases and phosphatases are in turn precisely regulated by reversible phosphorylation in response to a variety of signaling pathways. Importantly, MP is regulated by the kinases ROCK and ZIPK (dapk3), which can phosphorylate MP at the conserved inhibitory phosphorylation sites as well as phosphorylate MLC2 directly. In this work we characterize function of the zebrafish homolog of ZIPK. Oddly, ZIPK has undergone a unique divergence in sequence and function during the evolution of murine rodents. Thus, zebrafish and human Zip kinase are functionally more similar than are murine and human. Murine ZIPK localizes to the nucleus and requires an accessory protein, PAR-4 (prostate apoptosis response/pawr), to allow it to escape the nucleus and regulate the actin cytoskeleton. In contrast, we found that both human and zebrafish ZIPK localize to the cytoplasm and do not need additional factors to localize to the actin cytoskeleton. In addition, we determined that ZIPK is expressed maternally in zebrafish embryos and the expression drops off significantly but remains detectable and ubiquitous during zygotic-expression stages. Structure-function analysis of zebrafish ZIPK shows that unlike the mammalian orthologs its subcellular localization is not controlled by phosphorylation, however activating phosphorylation sites are conserved. In this work, taking a comparative biochemistry approach, we demonstrate that while the role of ZIPK in controlling MLC2 phosphorylation between mammals and zebrafish, significant mechanistic differences are scene in how the different orthologs are regulated.

586. Functional analysis of the novel autophagy proteins SCOC and FEZ1. ***Martina B Wirth***[1]*, Harold Jeffries*[1]*, Nicole Mc Knight*[1]*, Terje Johansen*[2]*, Sharon Tooze*[1]. 1) Secretory Pathways Laboratory, London Research Institute, Cancer Research UK, London, United Kingdom; 2) Molecular Cancer Research Group, Institute of Medical Biology, University of Tromsø, Tromsø, Norway.
Autophagy is a conserved and highly regulated catabolic pathway, transferring cytoplasmic components in autophagosomes to lysosomes for degradation and providing amino acids during starvation. Deregulation of autophagy is implicated in many human diseases (cancer, neurodegenerative disorders, inflammatory diseases), and there is a genuine need to learn more about the physiological and pathological role of autophagy in vertebrate organism, which will help to specifically target autophagy in disease. In a genome wide siRNA screen we identified SCOC (short coiled-coil protein), a small Golgi protein, as a novel positive regulator of starvation-induced autophagosome formation. SCOC interacts through FEZ1 with two key signaling complexes of the autophagic pathway: the ULK1 complex and Beclin1-PI3KC3 complex. The ULK1 (Unc-51-like kinase 1) protein kinase complex plays a central role in nutrient sensing and induction of autophagy in response to starvation. The Beclin1 class III phosphatidylinositol 3-kinase (PI3KC3) complex generates phosphatidylinositol 3-phosphate, which is an important signaling molecule for recruitment of other autophagy effector proteins during autophagosome formation. In vertebrates, both SCOC and FEZ1 are highly expressed in the developing nervous system. Moreover, worms mutant for SCOC (unc-69), FEZ1 (unc-76) or ULK1 (unc-51) exhibit an “uncoordinated phenotype” caused by defects in axonal elongation. Knockdown of ULK1 kinase in mouse brain also impairs axon outgrowth. Autophagy is a central homeostatic pathway for removal of damaged organelles and aggregated proteins and has been implicated in neurodegenerative diseases such as Alzheimer’s disease. Although autophagy is indispensible for nervous system development and neuronal health, relatively little is known about the precise mechanisms, which regulate autophagy in neurons. We recently started to use zebrafish as a model system to analyze the function of the novel autophagy proteins SCOC and FEZ1 in vertebrate development, tissue homeostasis and neurogenesis.

587. HIF-3 activates a unique transcriptional program in developing zebrafish embryos. ***Peng Zhang***[1]*, Ling Lu*[2]*, Cunming Duan*[1]. 1) Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Key Laboratory of Marine Drugs, Ocean University of China, Qingdao, China.
Hypoxia occurs during embryonic development and influences many developmental processes. Hypoxia also occurs in many pathological conditions and promotes tumor progression. Hypoxia-inducible factors (HIFs) are a family of transcriptional factors that play key roles in the transcriptional response to hypoxia. While the function of HIF-1 and its regulated transcriptional program are well studied, little is known about HIF-3 and its target genes. In this study, we have characterized and compared the transcriptional programs induced by Hif-3 and Hif-1 in zebrafish embryos. RNA samples isolated from zebrafish embryos expressing stabilized Hif-3a or Hif-1a were subjected to microarray analysis followed by RT-qPCR. Using a fold change > 2 and $P<0.05$ cutoff line, 155 Hif-3a up-regulated genes and 690 Hif-1a

up-regulated genes were identified. These genes can be categorized into 3 groups: a) genes that are regulated by Hif-3a only; b) genes that are regulated by Hif-1a only, and c) genes that are regulated by both Hif-1a and Hif-3a. Among the most highly Hif-3a up-regulated genes are caspase 6 (casp6l2), myosin (myhz2), zp3v2, claudin (cldn3l), calcium channel (cacng7b). GO and Pathway enrichment analyses suggest that Hif-3a and Hif-1a enriched genes in many overlapping processes/pathways, including glucose metabolism, ubiquitin mediated proteolysis, apoptosis, p53 signaling, and PPAR signaling. Hif-3a but not Hif-1a expression enriched genes involved in nitrogen metabolism, Jak-STAT signaling, and NOD-like receptor signaling. Hif-1a but not Hif-3a enriched genes involved in peroxisome, lysosome, VEGF signaling, insulin signaling, and MAPK signaling. To determine whether this Hif-3-induced transcriptional program is conserved in human cells, a human HIF-3a stabilized mutant was engineered and introduced to human HEK293 and U2OS cells. As in the case in zebrafish embryos, overexpression of human HIF-3a up-regulated similar genes in these human cells. These results suggest that Hif-3/HIF-3 activates a unique and evolutionarily conserved transcriptional program in response to hypoxia.

588. Vegf Promotes Endothelial Differentiation Of Arterial and Venous Progenitors By Modulating Etv2 / Etsrp Expression. ***Megan S. Rost***[1], *Saulius Sumanas*[1,2]. 1) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati OH, 45229, USA; 2) Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center / University of Cincinnati, 3333 Burnet Ave, Cincinnati OH, 45229, USA.
Major blood vessels acquire arterial-venous identity prior to the establishment of circulation. We have previously demonstrated that arterial and venous progenitors of the major axial vessels originate at distinct times and locations in a zebrafish embryo. Both Vascular Endothelial Growth Factor (Vegf) signaling and ETS transcription factors have been implicated in regulating arterial-venous specification and overall vascular endothelial differentiation. However, it is not clear how two different functions of Vegf and ETS factors are coordinated during embryonic vascular development. Here we explored the relationship between Vegf signaling and ETS transcription factor Etv2 / Etsrp function during endothelial differentiation and arterial-venous specification of the major axial vessels in zebrafish embryos. Our results argue that, in contrast to the current model, Vegf signaling does not specifically regulate arterial-venous specification but is required for the overall endothelial differentiation. In Vegf receptor inhibitor treated embryos, expression of both arterial and venous markers is downregulated. Conversely, Vegf overexpression results in the expansion of both arterial and venous markers. We further show that Vegf signaling modulates Etv2 expression. Etv2 expression is downregulated in Vegf inhibited embryos, and expanded in Vegf overexpressing embryos. Our results argue that Vegf regulates overall endothelial as well as arterial-venous differentiation by modulating expression of ETS factors including Etv2. We propose a revised model for arterial-venous differentiation where arterial-venous fates are determined not by the level of Vegf signaling but by the timing of endothelial differentiation.

589. Cdh5 promotes dynamic cell shape changes during angiogenic sprouting by coupling the cortical actin network to endothelial cell interfaces. ***Loïc Sauteur***, *Alice Krudewig, Lukas Herwig, Nikolaus Ehrenfeuchter, Anna Lenard, Markus Affolter, Heinz-Georg Belting.* Cell Biology, University of Basel, Biozentrum, Basel, Switzerland.
Organ morphogenesis requires coordinated cell behaviors to shape three-dimensional structures. We have recently described the morphogenetic processes that underlie blood vessel fusion and found that VE-cadherin (Cdh5) is essential for endothelial cell recognition at the onset of this process. In the present study we have analyzed the cell behaviors during sprouting angiogenesis and find that two distinct mechanisms are responsible for sprout outgrowth: tip cells show migratory behavior, where as stalk elongation results from cell elongation. To obtain a better understanding of these cell behaviors at the molecular level, we generated a targeted null-mutation in the *cdh5* gene. In the absence of Cdh5 we find: (1) inefficient contact formation between neighboring tip cells, which leads to a delay in anastomosis. (2) During sprout outgrowth the migratory behavior of tip cell is unaffected. (3) Stalk cell elongation is impaired and (4) stalk cells frequently lose connection to the leading tip cell. 3D reconstruction and quantification of stalk cell-cell interfaces reveal that Cdh5 is required for dynamic cell shape changes. Furthermore, the cortical F-actin network is disturbed in *cdh5* mutant stalk cells and expression of a truncated form of Cdh5, unable to bind F-actin, does not rescue the *cdh5* mutant phenotype. These findings support a model for angiogenic sprout outgrowth, where Cdh5 promotes cell shape changes by transmitting cytoskeletal forces, which deform intercellular surface areas and thus drive concerted stalk cell elongation.

590. Validation of Aneurysm Associated Genes in a Zebrafish Model. ***Quynh V. Ton***, *Saulius Sumanas.* Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.
Intracranial saccular (berry) aneurysms (IAs) are small berry or balloon-like defects in the wall of a major intracranial artery. The rate of mortality for affected patients is high; survivors will recover with major disability. In recent years, Genome Wide Association Studies have successfully identified novel genes associated with IAs. However, the population attributable risk of those novel genes remains quite small. To identify additional genes responsible for IA development, a Familial Intracranial Aneurysm Study utilizing whole exome sequencing (WES) was performed to identify single nucleotide polymorphisms associated with IA susceptibility in human patients. Since the transparent zebrafish embryos are advantageous for the evaluation of vascular development and stroke, we used the zebrafish embryos to determine if the top candidate genes found in patient tissues in the WES study are associated with aneurysms. Among the top genes in the prioritized gene-list, our data show that knockdown of 3 independent genes (COL22A1, PKD1, and HSPG2) by morpholino (MO) mediated gene knockdown each show hemorrhages in zebrafish embryos. We are generating genetic mutants to validate MO data and study hemorrhage phenotypes. We are also creating transgenic lines that over-express human wild type and mutant variants to determine the effect of the human mutant variants on the protein function. These analyses will contribute to our understanding of genetic causes of aneurysms that will aid screening to identify patients at risk and will enable development of new treatments.

591. Cerebral vascularization is a wreck without Reck. *Florian Ulrich[3], Jorge Carretero-Ortega[3], Evelyn Veliz[3], Belinda Sun[3], Valerie Pershad[3], Andrew Prendergast[2], Daniel Castranova[1], Brigid Lo[1], Makoto Kamei[1], Kameha Kidd[1], Kenna Shaw[1], David Raible[2], Brant Weinstein[1], **Jesus Torres-Vazquez**[3]*. 1) Section on Vertebrate Organogenesis, NICHD, Bethesda, MD; 2) Biological Structure, University of Washington, Seattle, WA; 3) Cell Biology, New York University, New York, NY.
Reck is a membrane-bound inhibitor of metalloproteinases (MPs), especially matrix ones (MMPs). MMPs are free and membrane-bound proteases for ligands, receptors and extracellular matrix (ECM) components. The ECM is a network of proteins, glycoproteins, proteoglycans and polysaccharides and forms the pericellular interstitial matrix and the basement membrane between tissues. It shapes tissues by modulating cell signaling, differentiation, adhesion, migration and invasion. Accordingly, Reck is pivotal for brain and tumor vascularization and both tumor invasiveness and metastasis. Yet, the cellular and molecular bases of Reck's effects are unknown. Zebrafish reck mutants (reck-) show MMP hyperactivity and a brain-specific vascularization deficit. The wild type (WT) hindbrain (HB) has extra- and intra-cerebral vessels. The former form first and from these (the Primordial Hindbrain Channels/PHBCs) the intra-cerebral Central Arteries (CtAs) sprout via angiogenic cell emigration. In reck- HBs, endothelial cell show proper abundance but abnormal distribution (hyperplastic PHBCs, no CtAs). Thus, reck-dependent endothelial cell emigration from PHBCs enables intra-cerebral vascularization. reck is expressed in the endothelium, and forcing WT reck cDNA expression in this tissue rescues reck-'s vascular defects. Moreover, WT endothelial cells transplanted into reck- hosts rescue the intra-cerebral vascularization deficit, indicating that reck acts in the endothelium. Brain angiogenesis requires reck and VEGF-A signaling and these two are linked. Expression of kdrl (VEGF-A receptor KDR/VEGFR2 ohnolog) is low in reck-'s PHBCs and forced endothelial kdrl expression rescues reck-'s brain angiogenesis deficit. RECK-depleted HUVECs have low KDR abundance and reduced VEGF signaling. Our data supports the model that Reck acts in the brain's endothelium by promoting responsiveness to VEGF-A signals via the modulation of kdrl/KDR expression.

592. Genetic and chemical dissection of the blood-brain barrier in zebrafish. ***Robyn A. Umans**[1,2], Hannah E. Henson[1,2], Bensheng Ju[1], Steven M. Finckbeiner[1], Michael R. Taylor[1]*. 1) Chemical Biology & Therapeutics, St. Jude Children's Research Hospital, Memphis, TN; 2) IPBS Graduate Program, University of Tennessee Health Science Center, Memphis, TN.
The blood-brain barrier (BBB) plays a vital role in both normal and pathological processes within the central nervous system (CNS). Yet, a comprehensive understanding of BBB development and maintenance has been hampered by difficulties in observing the BBB in vivo and in performing unbiased genetic and small molecule screens. Here we generated a transgenic zebrafish line to serve as an in vivo reporter of the BBB. We show that our transgenic line drives expression specifically in brain endothelial cells and not in the vasculature of peripheral tissues or circumventricular organs. Using time-lapse confocal microscopy, we find that barriergenesis (i.e. the initiation of BBB development) occurs immediately as new vessels sprout into the brain parenchyma. Using a combination of genetic and chemical tools, we demonstrate that canonical Wnt signaling, but not VEGF signaling, is essential for barriergenesis. These results indicate that CNS angiogenesis and barriergenesis occur simultaneously, but require distinct signals for proper formation. To expand upon these findings, we have initiated forward genetic and small molecule screens aimed to identify modulators of BBB function.

593. Combinatorial Control of Vascular Development by Transcription Factors Islet2 and Coup-TF. *Chiou-Hua Chen, Yu-Jheng Mou, Jia-Hong Wang, **Chang-Yi Wu***. Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan.
Multiple signaling pathways are required for the growth and patterning of blood vessels. Transcription factors that promoting vein and tip cell identities have remained largely unknown. The orphan nuclear receptor CoupTFII positively regulates vein identity in mice. We previously identified the LIM-Homeodomain transcription factor Islet2 required for specification of the vein and tip cell identity mediated by notch pathway in zebrafish, and coupTFII plays minor roles on that. In addition, we have found that loss of coupTFIb leads to a phenocopy of isl2 knockdown, suggesting CoupTFIb has a similar role in vascular development. To dissect the genetic interactions among the transcription factors isl2, coupTFIb and coupTFII, we use combinatorial knockdown, in-situ hybridization and rescue experiments. Double knockdown of isl2/TFIb, isl2/TFII and TFIb/TFII showed the enhanced defects in vasculature. We further showed that double knockdown of isl2/TFII results in the strongest suppression of flt4 expression suggesting that they likely act in parallel pathways. We also found Isl2 can regulate the expression of TFIb and TFII and overexpression of coupTF1b can partially rescue loss of isl2. Those data suggests a model where Isl2 functions upstream of CoupTF1b to promote vein and tip cell specification, while CoupTFII functions through a related, parallel pathway in vein differentiation. To reveal the molecular mechanisms how isl2 and coupTFIb promote the vascular development, we performed the microarray experiments to identify downstream targets of isl2 and/or coupTF1b. Our data suggests that TFIb and isl2 cooperatively regulate endothelial cell identity. We showed many target genes are spatiotemporal expressed in vessels during development. Promoter analysis suggests that most of targets contain one or more consensus binding sites of isl2 and/or coupTF. To further test the physical interaction between Isl2/TFIb and cis-elements of their targets, we performed luciferase assay. We showed isl2 and coupTFs cooperatively enhance gene expression. Together, we show that the interaction of the Islet2, CoupTF1b and CoupTFII transcription factors plays coordinated roles in vascular development in zebrafish.

594. Knockdown of ASD-linked genes SHANK3 and SYNGAP1 produces common embryonic phenotypes in zebrafish. ***Robert A. Kozol**[1], H. N. Cukier[2], B. Zhou[1], V. Mayo[2], S. De Rubeis[3], G. Cai[3], A.J. Griswold[2], P.L. Whitehead[2], J.L. Haines[2], J.R. Gilbert[2], M.L. Cuccaro[2], E.R. Martin[2], J.D. Baker[1], J.D. Buxbaum[3], M.A. Pericak-Vance[2], J.E. Dallman[1]*. 1) Dept of Biology, University of Miami, Coral Gables, FL; 2) HIHG, University of Miami Miller School of Medicine, Miami, FL; 3) Department of Psychiatry, Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY.
Autism Spectrum Disorders (ASD) are a complex of neurodevelopmental diseases that affect ~1% of children in the United States (Centers for Disease Control, 2012) Genetic mutations in a diverse array of genes have been implicated in ASD, but currently only 15-30% of cases have known genetic etiologies. ASD gene validation and functional annotation have relied heavily upon animal modeling, much of which has focused on post-embryonic stages associated with synaptogenesis. By contrast, embryonic stages have largely been unexplored, leaving

a gap in our current understanding of ASD disease gene function. In this study we explore embryonic roles of the ASD-linked orthologs *SHANK3*, a scaffolding protein, and *SYNGAP1*, a synaptic GTPase activating protein in zebrafish. Both *SHANK3* and *SYNGAP1* are known to play essential roles in the post-synaptic density of mature excitatory synapses, however both show broader neuronal expression during embryonic vertebrate development. To study the embryonic roles of *shank3* and*syngap1*, we targeted these genes for knock-down with splice-inhibiting morpholinos (MO). Morphant embryos displayed disrupted escape behaviors by 48 hours post fertilization (hpf), which included unproductive swimming and seizure-like phenotypes. Morphological phenotypes included hydrocephaly and microcephaly, and significant cell death at 24-28 hpf. Cell death was partially rescued by *shank3a* MO and *syngap1b* MO co-injections with full length SHANK3 and SYNGAP1 human mRNAs, respectively. This rescue suggests that both shank3 and syngap1 normally help to protect against widespread neuronal cell death during embryonic development. Our study implicates shank3 and syngap1 in similar biological processes and demonstrates how zebrafish can help elucidate novel embryonic roles that may contribute to the development of symptoms associated with ASD.

595. Zebrafish as a genetic model for skull and suture development. ***Katrin Henke***, *M. Brent Hawkins, Matthew P. Harris.* HMS Genetics, BCH Orthopaedics, Boston, MA.
The establishment of the form of the adult cranium requires tight coordination of growth and differentiation of the many independent skeletal elements of the skull during post-embryonic development. For example, early fusion of the fibrous connections between the calvarial bones or craniosynostosis, is a common disorder in children that besides causing deformations of the skull also constricts brain growth leading to mental retardation and other complications. Up to date, only very little is known about the genes controlling suture development and retention of patency, therefore the mechanisms underlying precocious suture fusion remain unclear. The zebrafish skull shares many of the same structures with the human skull, including sutures that form between growing bones. Therefore, dissecting the genetic basis of skull development in zebrafish can aid in understanding human skull development and disease etiology. In two large-scale ENU-mutagenesis screens, we have identified both recessive and dominant mutants showing alterations in skull morphology. These mutants can be grouped into different phenotypic classes based on their suture morphology. Besides mutants with altered skull morphology but normal cranial suture development, we identified mutants showing premature suture fusion, mutants with additional sutures and mutants with miss-patterned calvarial bones. We are mapping these mutants using next-generation sequencing techniques developed in the lab and currently have identified several genes regulating suture patency. Interestingly, identified genes from these screens regulate ECM maturation and cell-ECM signaling suggesting a role of post-translational regulation of the ECM in control of suture patency. To test this hypothesis, we are analyzing the composition of the ECM in wild type and mutants using liquid chromatography-mass spectrometry (LCMS) to test if miss-regulation of post-translational modification is a common attribute among defective sutures. With the analysis of our mutant collection, we hope to get a better understanding of the complex interactions between tissues that underlie the attainment of form and function of the skull.

596. IGF1 stimulate formation and growth of previtellogenic follicles in ovaries of zebrafish Danio rerio cultured in vitro. ***Pancharatna A Katti***, *Prasad A Deshpande.* Dept of Studies in Zoology, Karnatak University, Dharwad, India.
Ovaries from adult (body size: 27 ± 1 mm) zebrafish, (Danio rerio) were cultured in vitro, in chemically defined medium supplemented with different doses (0.1, 0.5, 1.0, 5.0 and 10 ng/ml) of recombinant human insulin like growth factor 1(rhIGF1), in order to investigate the intraovarian regulation of formation and growth of previtellogenic follicles in the fish ovary. Ovarian cultures set in triplicate for each dose of IGF1 were maintained under aseptic conditions at 22º C for 15 days. Corresponding controls and initial controls were used for comparison. On 16th day, the ovaries were fixed in Bouin's fluid and processed for paraffin embedding; sections of 3 mm thick were cut and stained with hematoxylin and eosin. Serial sections were carefully observed under microscope and follicles were quantified. The results reveal that, at the beginning of the experiment, initial control ovaries contained all types of follicles i.e. newly formed follicles, previtellogenic follicles in primary growth phase and cortical alveolar stage, follicles in vitellogenic growth phase and large post vitellogenic follicles. In 15 day in vitro cultured ovaries, all large vitellogenic follicles underwent atresia, while, a few cortical alveolar follicles survived; but previtellogenic follicles were unaffected. In IGF1 exposed ovaries, previtellogenic follicle number was not only greater compared to controls, but their growth was stimulated and most of them attained yolk vesicle stage, although the culture medium was not supplemented with any gonadotropin or steroid hormone, Our results suggest that, local ovarian factors may be involved in the recruitment and early growth of follicles in fish ovary.

597. BMP signaling in normal and abnormal cranial suture development. ***Jacqueline C Simonet***, *Shannon Fisher.* Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
Craniosynostosis (CS) or premature closure of the cranial sutures is a common congenital defect, which can occur as part of several syndromes, but more often is nonsyndromic. Several signaling pathways have been implicated in syndromic forms of CS, including BMP signaling. Bmp2 is expressed in osteogenic areas during suture development and inhibition of Bmp impairs suture closure while increased Bmp signaling leads to CS. In humans, activating mutations in the transcription factor MSX2, a target of BMP signaling, cause Boston type CS, while haploinsufficiency leads to delayed suture closure. However, for the most common subtype, nonsyndromic sagittal CS, the genetic basis is not known in most patients. Genome-wide association studies (GWASs) can identify genetic risk factors in complex diseases of unknown etiology. Most single nucleotide polymorphisms (SNPs) mapped by GWASs are not associated with coding sequence changes in nearby genes, implicating mutations in regulatory elements. However, in most cases regulatory regions are not known, hampering efforts to identify the causal mutations. Recently the first GWAS for CS, on nonsyndromic sagittal CS patients, identified two risk-associated loci, one near BMP2 and one within BBS9. The susceptibility locus near BMP2 likely indicates a regulatory element controlling BMP2 expression during cranial suture development. While BBS9 has no obvious biological link to CS, it has a conserved syntenic association with BMPER, which regulates Bmp signaling. We hypothesize that the risk locus within BBS9 is associated with an

enhancer for BMPER. Therefore, we are evaluating conserved sequences within the 167kb association region for regulatory activity in transgenic zebrafish. We are using a set of vectors incorporating FC31 integrase, to allow quantitative assessment of enhancer activity in a single genomic context. We will thus be able to evaluate candidates for the causal mutation by determining the effect of disease related SNPs on enhancer activity. This approach will also be generally useful to find enhancers associated with disease risk in the large majority of GWASs in which no coding sequence changes have been identified.

598. Zebrafish-based Chemical Biology of In Vivo Fluorescent Imaging for Leukemia Stem Cells. ***Toshio Tanaka***[1], *Beibei Zhang*[1], *Yasuhito Shimada*[1], *Noriko Umemoto*[1], *Yuhei Nishimura*[1], *Tsuyoshi Nomoto*[2], *Taichi Shintou*[2], *Takeshi Miyazaki*[2]. 1) pharmacogenomics and Systems Pharmacology, Mie University, Tsu, Japan; 2) Corporate R&D Headquarters, Canon Inc., Tokyo, Japan.
Increasing evidence indicates that cancer stem cells are initiators of the occurrence, development and recurrence of malignant tumors. The ablation of the leukemia stem cells is necessary to destroy the malignant cell population permanently. Due to the very small number of leukemia stem cells in total leukemia cell population in xenotransplantation studies in vivo and the difficulties in functionally and pathophysiologically replicating clinical conditions in cell-based experiments in vitro, the strategy for finding new anti-leukemia stem cell drugs has been still immature. Recently, we reported the quantitative phenotyping-based leukemia stem cell xenograft zebrafish screening system to evaluate the selective human leukemia stem cell inhibitors in vivo (PLOS ONE 9(1):e85439,2014). In this study, we conducted the chemical screening from a fluorescent chemical library using leukemia stem cells xenograft zebrafish, and found that the fluorescent compound DiOC5(3) (3,3'-dipentyloxacarbocyanine iodide) selectively visualized leukemia stem cells and suppressed their proliferation in vivo and in vitro. DiOC5(3) accumulated mitochondria through organic anion transporter polypeptides which overexpressed in the plasma membrane of leukemia stem cells, and induced ROS overproduction by the inhibition of mitochondrial NADH oxidase with inactivation of NF-kB signaling selectively. In addition, DiOC5(3) had no obvious toxicity to human umbilical blood CD34+ progenitor cells and normal zebrafish. Transcriptome analysis revealed that selective leukemia stem cell inhibition mechanisms of DiOC5(3) mainly depended on the gene down-regulation of cell cycle, DNA replication, DNA repair, and gene up-regulation of cellular stress, apoptosis, cell differentiation. In summary, DiOC5(3) is proved to be a new type of anti-leukemia stem cell drug with availability for diagnostic imaging and therapeutics in a single fluorescent chemical. Zebrafish-based and phenotype-driven chemical screening could be a promising approach in drug discovery against leukemia stem cells.

599. PCBs & Aroclors disrupt development through the AhR & ER pathways. ***Corinna Singleman***[1,2], *Elise Harrison*[1], *Nathalia Holtzman*[1]. 1) Biology, Queens College CUNY, Queens, NY; 2) CUNY Graduate Center, 365 Fifth Ave, New York, NY.
Polychlorinated biphenyls, PCBs, were used in industry as mechanical lubricants and insulators, and as additives in pesticides and paints for decades due to their high stability under stresses. PCBs have become a significant environmental hazard as they were disposed of in local waterways and soils. PCBs bioaccumulate in fatty tissues, often causing developmental delays, reproductive problems and cancer. General Electric dumped 1.3 million pounds of PCBs into the Hudson River. These PCBs can now be found in the sediments around New York City and have made their way into people. PCBs are a classified by chemical structure as either coplanar or non-coplanar. These two main classes of PCBs are thought to differentially effect biological processes; typically, coplanar PCBs have a high binding affinity to aryl hydrocarbon receptor (AhR), while some non-coplanar PCBs have been shown to act as a ligand for estrogen receptor (ER). For example, the coplanar PCB 126 causes heart defects in fish however, the consequence of PCBs found in the Hudson River are poorly studied. We have defined the developmental effects of four PCB mixtures, Aroclors, dumped into the Hudson River by exposing zebrafish embryos to four environmentally relevant PCB concentrations for the first five days of development. We observed abnormal gross morphological changes in developing embryos and disrupted organ development. Cardiac development is retarded; specifically we see failure of cardiac looping and chamber ballooning. In addition, gastrointestinal development is disrupted, the pancreas contains excess insulin positive cells and the liver is smaller. Utilizing Tg(5xERE:egfp) fish (ER pathway activation) and CYP1A immunohistochemistry (AhR pathway activation) we are beginning to decipher the biological pathways affected by PCB and Aroclor exposure to zebrafish.

600. Assigning biological function using high throughput sequence based technology. ***Neha Wali***, *John E. Collins, Ian Sealy, Richard J. White, Christopher M. Dooley, Catherine Scahill, Samantha Carruthers, Zsofia Pustzai, Ian Packham, Peter Clarke, Jorge Zamora, Nicole Staudt, Amanda Hall, Ross N.W. Kettleborough, Elisabeth M.Busch-Nentwich, James Morris, Jeff Barrett, Derek L. Stemple.* Wellcome Trust Sanger Inst, Cambridge, United Kingdom.
The emergence of high throughput sequencing has paved the way for rapid identification of inherited and *de novo* disease causing mutations, many of which are contained in genes with little to no existing functional information. The Zebrafish Mutation Project (ZMP) aims to determine detailed biological function by first assessing morphological phenotypes arising from our on-going loss of function screen. This is followed by a new sequence based single embryo transcript counting method, the Differential Expression Transcript Counting Technique (DeTCT) which allows us to assign biological function to these genes by quantitative analysis of alterations in transcript abundance at a genome wide level. The gene lists generated by our method provide an insight into the molecular changes underlying morphological phenotypes as well as uncover molecular phenotypes for knockouts with no obvious morphological phenotype. These gene lists, along with images and ontology based phenotype annotations are available as an interactive table and for download on our website at http://www.sanger.ac.uk/resources/zebrafish/zmp/. We have begun to analyse the data generated and to build pathway-specific genetic networks. This has revealed molecular profiles of knockouts in previously uncharacterised genes, identifying novel genes affecting overlapping developmental pathways. We also aim to use the DeTCT pipeline to define developmental stages based on transcriptional signatures and also to identify pathways and novel targets for a variety of compounds known to affect early development. We will present the methodology and examples of gene lists generated to highlight the utility of the DeTCT pipeline.

601. The receptor for estrogen hormone alters the pacemaker potential of embryonic heart cells during development. ***Monika Andersson-Lendahl***, *Kent Ivarsen.* Karolinska Institutet, Department of Cell and Molecular Biology (CMB), Stockholm, SWEDEN.
Heartbeating is determined by the electric cardiac conductive system (CCS) and the pacemaker cell membrane potential in the sinoatrial node is coordinating the rhythm of the beats. We have investigated a role of estrogens in the regulatory network of the CCS in the developing heart in vivo. Zebrafish embryos were subjected to different concentrations of the natural hormone 17b-estradiol (E2) and embryos were analyzed for survival, morphology and heartbeat pace. Two developmental stages were analyzed, 30 hours post fertilization (hpf) and 48 hpf. At 30 hpf the E2 subjected embryos show a heartbeat pace of 30% above that of control embryos. At 48 hpf the control embryos show a heartbeat rate that is higher than the pace of the E2 treated embryos (80% of the control pace). Although an elongated heart with heart edema was present in the ligand treated embryos, the synchronicity of the developmental stages were not affected by E2 and arrythmia was not detected in the embryos. When embryos were co-subjected to both E2 and the E2 antagonist (ICI 182.780) the frequencies of the heart rates were reverted to control levels, both at 30 hpf and at 48 hpf. In addition to the altered heartbeat rates, all E2 subjected embryos showed a tail phenotype that was not rescued by the E2 antagonist. Using estrogen receptor morphants we confirm that the signals governing the pace of heart contractions are sensitive to the levels of estrogen receptor expression. The overall results show that myogenic cells of the CCS, but not the myogenic cells of tail (skeletal) are regulated through a pathway in which estrogen receptors are regulated through a pathway in which estrogen receptors are engaged.