SPRING 2018 MID-ATLANTIC REGION ZEBRAFISH MEETING

Photo Courtesy of:
Michael Grillo; PhD
Stella Laboratory, Penn State University,
Crystal Het GCaMP6f zebrafish larvae

Friday
April 13th

Penn State
College of Medicine

MARZ @ HERSHEY
Penn State Zebrafish Functional Genomics Core

- Twin 16-rack recirculating aquaria
- Isolated quarantine room
- 4 microinjection stations
  - Femtojet microinjector
  - Discovery V8 microscope
- 2 Fluorescent light-tight imaging booths
  - Zeiss AxioZoom V16 with ApoTome.2
  - Olympus MVX10
- http://med.psu.edu/core/animal-services
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Congratulations to Spencer Katz for winning our MARZ logo design contest and coming up with this beautiful logo! Spencer is working on his MD/PhD at Penn State University, College of Medicine.
Welcome to Hershey – the sweetest place on earth!

We at Penn State College of Medicine thank you for participating in this 2018 Spring Mid-Atlantic Region Zebrafish meeting, brings together 128 researchers from 28 institutions giving a total of 13 oral and 33 poster presentations.

We thank the Penn State College of Medicine for their support in hosting this meeting and Jake Gittlen Laboratories for Cancer Research for providing tremendous help in organizing this event. We also thank and acknowledge our sponsors, whose generous support has enabled us to provide sustenance for this meeting!

We hope that you will have a great meeting, productive engagements and networking with the wonderful zebrafish community represented here!

2018 Spring MARZ Organizing Committee
We are grateful to our sponsors who funded this meeting!
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<td>Simplet-dependent regulation of β-catenin signaling influences skeletal patterning downstream of Cx43, Shashwati Bhattacharya, Lehigh University</td>
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<td>The ciliary marginal zone: An in vivo model for understanding epigenetic regulation of adult stem cell maintenance, Krista Angileri, U of Pittsburgh</td>
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<td>Somatic Loss of the B Subunit of DNA polymerase α Causes a Range of Tissue-Specific Cellular Changes Including Nuclear Atypia and Cell Death in Zebrafish, Alex Lin, PSU</td>
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<td>Digits and fin rays share common developmental histories, Tetsuya Nakamura, Rutgers</td>
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<td>Molecular regulation of vascular smooth muscle cell recruitment to arteries during development, Amber Stratman, NICHD/NIH</td>
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Teresa Porri is the CT manager at the Cornell Biotechnology Resource Center Imaging Facility. She earned a BA in Chemistry from Ithaca College and a Ph.D. in Materials Science and Engineering from the University of Wisconsin-Madison, where she studied the effect of nanopatterned surfaces on cell behavior. The skills in interdisciplinary research that she gained in her graduate studies have translated well to Cornell University’s scientific core facilities, where she has worked for the past 10 years, and where she enjoys working on a wide variety of research topics, engaging in outreach activities, and helping all sorts of science to get done more efficiently.

Abstract

"What's in the cabinet right now?": Exploring the World using Non-Invasive X-ray Microscopy

CT (computed tomography) is a well-known technique in medicine, where its ability to yield 3D information non-destructively using x-ray images is highly valued. However, its utility in other fields is still ripe for exploration. In this talk, I will give some background on CT and x-ray microscopy and provide examples of the wide-ranging scientific fields to which CT imaging at the BRC Imaging Facility has made contributions, including materials science, vertebrate and invertebrate biology, biomedical engineering, food science, plant science, computer science, fiber science, art restoration, electronics, and archaeology.
Oral Presentation Abstracts

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O1: A comprehensive map and comparative analysis of cis-regulatory elements in the zebrafish genome

Hongbo Yang¹, Tingting Liu¹, Yu Luan¹, Yanli Wang¹, Bo Zhang¹, Glenn S. Gerhard², Darius Balciunas³, Keith C. Cheng¹, Feng Yue¹

1) Dept. of Biochemistry and Molecular Biology, Penn State University College of Medicine, 2) Dept of Biology, Temple University, 3) Dept of Medical Genetics and Molecular Biochemistry, Temple University

Zebrafish has been widely used for the study of human diseases, as ~70% of the protein-coding genes are conserved between the two species. Moreover, zebrafish embryos are transparent and thus can serve as an ideal model for genetic studies in animal development. Surprisingly, the functional annotation of zebrafish genome itself has been severely lagging when compared with other model systems such as mouse and drosophila. Here we took a similar approach adopted by the ENCODE and Roadmap Epigenomics projects, and performed RNA-Seq, ChIP-Seq and HiC for several histone modifications to generate a comprehensive map of transcriptomes and regulatory elements in a variety of zebrafish tissues, including brain, heart, liver, skeletal muscle, kidney and two embryonic tissues. We predicted over 100,000 cis-regulatory elements in the zebrafish genome, the most comprehensive functional annotation effort in zebrafish so far to our knowledge. We also identified tissue-specific and developmental stage-specific regulatory elements. By comparing the data generated by the ENCODE and Roadmap Epigenomics projects, we also defined a set of functionally conserved and species-specific regulatory sequences among zebrafish, mouse and human. We also generate the 3D genome map of zebrafish brain and muscle and found that Topological association domain (TADs) are pretty conserved from fish to human. In summary, we generated a great genomics/epigenomics resource for the functional annotation in the vertebrate genomes and further expanded the value of zebrafish as a model of human disease.
Fibrodysplasia ossificans progressiva (FOP) is a rare and debilitating human genetic disorder that perturbs skeletal development and induces heterotopic ossification. Classical FOP is caused by a single nucleotide substitution in the BMP/TGFβ cell surface receptor, ACVR1 (617G>A, R206H). This mutation results in over-activation of receptor signaling through the Phospho-Smad1/5 (pSmad1/5) pathway. However, the mechanism through which the mutant receptor confers enhanced signaling activity remains uncertain. To assay for mutant ACVR1 activity, we used zebrafish embryonic dorsoventral (DV) patterning, which is established by a gradient of BMP signaling activity that specifies ventrolateral cell fates. We confirmed that ACVR1-R206H misexpression causes over-activation of pSmad1/5 activity and ventralization of zebrafish embryos. We tested several rare ACVR1 mutations of FOP for signaling activity in the zebrafish embryo assay and found similar results. Recent studies suggest that ACVR1-R206H and other FOP variant mutant receptors may have altered ligand affinity compared to WT ACVR1. We confirmed that BMP ligand enhances pSmad1/5 signaling through ACVR1-R206H. Surprisingly, Activin A, a ligand that normally binds ActR1 and signals through pSmad2/3, also enhances pSmad1/5 signaling by ACVR1-R206H. In addition to testing ligand enhancement, we examined if other receptors are required with ACVR1 to induce signaling. We found that BMPR1, a receptor normally required for pSmad1/5 signaling and DV patterning in the zebrafish, is not required for pSmad1/5 over-activation by ACVR1-R206H or the variant mutant ACVR1-G328R. These data suggest that BMPR1 is not required for the pathogenesis of FOP. These and further studies of the signaling interactions of ACVR1-R206H will allow for identification of novel therapeutic targets to treat FOP and give us unique insight into how this fundamental cell signaling pathway functions in development.
O3: Genetic regulate on of myoblast fusion in zebrafish

Yufeng Si, Mengxin Cai, Jun Shi and Shaojun (Jim) Du
Institute of Marine and Environmental Technology, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore

Myoblast fusion is essential for skeletal muscle development, maintenance and regeneration. Defective myoblast fusion leads to muscular diseases in humans. Recent studies demonstrated that junctional adhesion molecules Jamb and Jamc, and a novel membrane protein Myomaker play vital roles in myoblast fusion in early muscle development. However, it is not clear whether genetic interactions exist between Jamb, Jamc and Myomaker in regulating myoblast fusion during early muscle development, and whether they function in myoblast fusion during muscle growth in juvenile and adult myofibers. To determine the genetic interaction and function of Jamb, Jamc and Myomaker in fish muscle development and growth, single mutant of jamb, jamc or myomaker and double mutant of jamb;jamc or jamb;myomaker were obtained and generated by CRISPR in zebrafish. All these mutants showed defective myoblast fusion in fast muscles during early myogenesis. However, jamb, jamc single mutant and jamb;jamc double mutant grew normally as wild type sibling. Single fiber analysis showed that myofibers from jamb, jamc or jamb;jamc adult mutant contained multiple nuclei as WT control. Temporal analysis revealed that myoblast fusion starting around 5 dpf in jamb or jamc single mutant. In contrast, myomaker mutant showed no myoblast fusion when analyzed on 5, 7, 11 dpf. Unlike jamb and jamc mutant fish that grow normally as WT control, myomaker single and jamb;myomaker double mutants showed partial lethality with approximately 40% of homozygous mutant survival to adulthood. The myomaker single and jamb;myomaker double mutants were smaller in size and weighted approximately 1/3 of the WT adult sibling. Histological analysis by HE staining revealed that myomaker mutant had fewer number of myofibers compared with the WT adult sibling. Moreover, the mutant myofibers were smaller in size/diameters. Single fiber analysis identified multiple nuclei in myofibers from myomaker or myomaker;jamb adult mutants. However, the number of nuclei was significantly lower in the mutant fibers compared with that in the WT control. In addition to the muscle defect, large number of intramuscular adipocytes was detected in skeletal muscles of myomaker mutant. Collectively, these studies demonstrate that although Jamb and Jamc are required for myoblast fusion in early myogenesis, loss of Jamb and Jamc has no effect on myoblast fusion at later stages in fish larvae and juvenile during muscle growth, suggesting that other redundant factors may be involved. Consistent with this idea, loss of Myomaker severely compromises myoblast fusion and muscle growth.
Hair cell death can be caused by a number of therapeutic medications and hearing loss is often a dose-limiting side effect of these medications. We recently found that mutations in a number of cilia-associated genes lead to resistance in aminoglycoside-induced hair cell death. Of those genes two different retrograde intraflagellar transport (IFT) mutants, motor gene dync2h1 and IFT-A complex gene wdr35, showed comparable levels of hair cell protection but differing levels of reduced aminoglycoside uptake into hair cells (Stawicki et al., 2016). To further investigate whether different classes of retrograde IFT genes differently affect aminoglycoside entry into hair cells we investigated mutants in the retrograde IFT motor gene, dync2li1 and three additional IFT-A adaptor genes, ift122, ift140, and wdr19. We found that mutations in all these genes lead to reduced hair cell death in response to aminoglycosides as well as a significant reduction in aminoglycoside and FM1-43 entry into hair cells comparable to what had previously been seen in dync2h1 mutants. To test whether dync2h1 and wdr35 are really protecting hair cells via different mechanisms we generated double mutants and found that there was no additive protection in these animals suggesting these genes may be playing similar roles in aminoglycoside-induced hair cell death. Lastly, it had previously been suggested that anterograde IFT genes were important for the localization of usher gene products in hair cells (Blanco-Sahnnez et al., 2014). To see if this was also the case for retrograde IFT genes we investigated the localization of fluorescently conjugated versions of Harmonin and SANS in dync2h1 mutants. We found that both these constructs were able to normally localize to the stereocilia in this mutant background.
Many genes thought to play essential roles in regeneration are expressed in multiple tissues and at different time points during development. The ability to mutate such pleiotropic genes in a spatiotemporally restricted manner is essential to understanding their functions during regeneration; thus, conditional mutants are necessary. Using the CRISPR/Cas9 system, we have developed a straightforward and highly efficient method to generate conditional alleles by sequential integration of loxP sites using single stranded oligonucleotide templates. We have successfully generated conditional alleles for two genes, tbx20 and fleer, and demonstrated temporal regulation of excision by utilizing tamoxifen-inducible CreERT2 recombinase. We have also integrated single loxP sites into two additional genes, aldh1a2 and tcf21, further demonstrating the ease and broad applicability of our approach.
Next-generation imaging modalities present a tremendous challenge: how to elegantly disseminate and fully communicate important concepts derived from very detailed, but often unwieldy, 2-dimensional and 3-dimensional images. Solutions to this shared challenge are needed in order to enhance biomedical research, as well as enrich educational endeavors. Extreme detail and scale are inherent to high-resolution data, resulting in massive file sizes (routinely exceeding 100 gigabytes each). Most computers cannot open files of this magnitude, making the sharing of such data a real-world challenge. Down-sampling the resolution of this data, an often utilized approach, results in fuzzy images that can lead to misleading conclusions. Additionally, cropping reduces file size for high-magnification fields, but these images lack anatomical context and proper sampling necessary for full understanding. In short, there is a pressing need for well-curated, web-based collections of high-resolution images that are accessible to fully interactive exploration, efficient transfer of expert knowledge, and maintenance of context. In the era of big data analytics, a web-based visualization platform is essential for wide dissemination of high-resolution imaging datasets and associated analysis files as exemplified by the Harvard Z-Brain Atlas and Allen Brain Institute mouse connectivity database. Such an infrastructure is needed for effective information exchange of big data between researchers, which is not possible via regular publications. Herein, we present a web-based community resource aimed at making scientific visualizations for next generation imaging modalities highly accessible to all end-users.
O7: Studying the origin and function of novel brain vascular-associated cells

Marina Venero Galanternik, Ryan D. Gober, Daniel Castranova, Andrew Davis, and Brant M. Weinstein
Division of Developmental Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, 20892

The meninges are an external enveloping connective tissue that encases the brain, homes cerebrospinal fluid, act as a cushion against trauma, nourishes the brain via nutrient circulation, and removes toxic waste. Despite its importance, the cell types present in the meninges and the function and embryonic origins of this tissue are still not well understood. We describe a novel perivascular cell population closely associated with blood vessels on the zebrafish brain meninges. Based on similarities in their morphology, location, and highly unusual scavenger behavior, these cells appear to be the zebrafish equivalent of mammalian “Fluorescent Granular Perithelial cells” (FGPs), macrophage-like cells about which very little is known that likely play important roles in brain function and in a variety of CNS pathologies, including Alzheimer’s disease. Using RNA-seq of FACS-sorted FGPs and single-cell profiling of cranial cells, we show that despite their macrophage-like morphology and their perivascular location these cells are molecularly most similar to lymphatic endothelial cells, and lineage tracing and time-lapse imaging demonstrate that these unusual cells transdifferentiate from endothelial cells lining blood vessels of the optic choroid vascular plexus deep inside the brain, before migrating to the brain surface. Using forward-genetic screening of transgenic zebrafish for ENU-induced recessive mutations, we recently identified a mutant that appears to be specifically deficient in FGPs, providing us a genetic model that we are using to further explore the likely very important functional role of these cells. Our findings thus far provide the first report of a non-vessel forming, perivascular cell population in the brain that emerges by transdifferentiation from vascular endothelium.
Joints are required for flexibility in the skeletal system. The process of joint formation and correct joint placement in the vertebrate system are not well understood. We use the Zebrafish caudal fin ray which is a rich source of joints, to study joint morphogenesis. Mutations in cx43 cause the sof b123 phenotypes, which include reduced fin length, reduced cell proliferation, and premature joint formation (i.e. short fin ray segments). We have shown that Cx43 suppresses joint formation by negatively influencing evx1 expression, a transcription factor required for joint formation. Here, we provide further insights into how Cx43 influences evx1 transcription. First, we find that simplet (smp) is downstream of cx43. Morpholino-mediated knockdown of smp recapitulates the sof b123 phenotypes of reduced regenerate length and reduced segment length, and we find evidence for synergy between cx43 and smp. Moreover, knockdown of smp also increases the evx1 expression, similar to sof b123/+ and cx43 knockdown. The smp gene is homologous to human FAM53B, and encodes the protein Simplet which has a nuclear localization signal and acts as a regulator of transcription and cell proliferation. Previous studies have shown that Smp is required for the nuclear localization of β-catenin and hence plays an important role in canonical Wnt signaling. Indeed, β-catenin activity is reduced in both sof b123 mutants and following Smp knockdown in regenerating fins. We further show that blocking canonical Wnt signaling results in a synergistic reduction in segment length in sof b123/+ heterozygotes. We conclude that both smp and β-catenin function in a common molecular pathway with cx43 to influence evx1 expression. Together our findings suggest that cx43 regulates evx1 expression by influencing the nuclear localization of β-catenin.
O9: The ciliary marginal zone: An in vivo model for understanding epigenetic regulation of adult stem cell maintenance

Krista M. Angileri and Jeffrey M. Gross
University of Pittsburgh School of Medicine

The zebrafish retina contains a population of retinal stem cells (RSCs) that reside within the ciliary marginal zone (CMZ). RSCs in the CMZ generate all seven retinal cell types and remain proliferative throughout life. DNA methyltransferase 1 (dnmt1), expressed within the CMZ, copies the DNA methylation pattern from parent to daughter strands during DNA replication. Loss of dnmt1 function results in the loss of the CMZ by 6 days post fertilization (dpf). We hypothesize that dnmt1 activity is required to maintain RSCs in the CMZ. To determine the onset of the CMZ phenotype, retinal cell numbers were quantified between 3-5dpf. The dnmt1-/- CMZ was reduced 77% by 5dpf when compared to sibling controls. The dnmt1-/- CMZ displays an 85% reduction in the proportion of BrdU+ cells at 5dpf. Preliminary gene expression analyses at 4dpf demonstrate dnmt1-/- RSCs maintain their progenitor-like domains (col15a1b and dnmt1), while genes required for cell cycle progression and differentiation (cyclinD1 and atoh7 respectively) are reduced. Additionally, the cell cycle inhibitor, cdkn1c, was ectopically expressed by RSCs in the dnmt1-/- CMZ, suggesting that cells are exiting the cell cycle prematurely. Cellular birth-dating experiments indicate RSCs do not differentiate properly. Increased ripk3+ staining at 5dpf indicates activation of the necroptotic cell death pathway. Treatment with the necroptosis inhibitor, Necrosulfonamide, rescues the dnmt1-/- CMZ phenotype. Additionally, loss of DNA methylation is known to upregulate retrotransposon activity, and we are analyzing this in the CMZ using a GFP-tagged human LINE1 retrotransposon construct.

These results support a model in which dnmt1 function is required to maintain RSC stemness to promote self-renewal within the CMZ; without dnmt1 activity, RSCs stall during the cell cycle and undergo necroptosis. Ongoing experiments will identify gene regulatory networks required for RSC maintenance through comparative transcriptome analyses of RSCs in the CMZ of wildtype and dnmt1-/- retinas.
O10: Somatic Loss of the B Subunit of DNA polymerase α Causes a Range of Tissue-Specific Cellular Changes Including Nuclear Atypia and Cell Death in Zebrafish

Alex Y. Lin, Georgia Thomas & Keith C. Cheng, Penn State College of Medicine

Despite the importance of nuclear atypia as a diagnostic feature of cancers and precancers, its genetic origin is poorly defined. The zebrafish huli hutu (hht) mutant has an obvious small eye and head phenotype and histologically-defined cellular change across a wide range of tissues, including nuclear atypia in the gut. Positional cloning revealed a loss-of-function mutation in the B subunit of the essential, replicative DNA polymerase α as responsible for the hht phenotype. The 5-7 day survival of hht zebrafish stands in striking contrast with the lethality of corresponding mutants in yeast and Arabidopsis beyond the first cell cycle. This difference is explained by the presence of wild-type maternal pola2 mRNA in mutant embryos at 6 hours-post-fertilization (hpf), followed by its progressive depletion. This supports a model in which initial survival progressing through increasingly abnormal development is driven by the presence of early polymerase α activity that has no genomic source for new wild-type transcripts. These results, together with the ability to phenocopy hht using inhibitors of DNA synthesis, indicate that progressive deficiency in DNA polymerase-mediated DNA synthesis is a potential source of the nuclear atypia seen in cancer.
O11: Using optogenetics to probe the limits of ERK signaling in the early zebrafish embryo

Aleena L. Patel¹, Rebecca D. Burdine², Jared E. Toettcher², Stanislav Y. Shvartsman¹,²
¹Chemical and Biological Engineering Department, Princeton University, Princeton, NJ 08544 ²Molecular Biology Department, Princeton University, Princeton, NJ 08544

The Extracellular Signal-Regulated Kinase (ERK) pathway is one of many signaling systems that regulate emerging patterns of cell fates during animal embryogenesis. Mutations in pathway components disrupt normal embryogenesis by altering the strengths, spatial extents, and durations of pathway activation. In the early zebrafish embryo, abnormally strong signaling activity caused by constitutively active mutant forms of the penultimate molecule in the ERK pathway, MEK, leads to an elongated aspect ratio of the yolk, at 11 hours post fertilization. Moreover, this phenotype is quantifiable, and is ranked with severity of the MEK mutation. What developmental time window is most sensitive to aberrant ERK activity in the early zebrafish embryo? To prescribe precise spatiotemporal patterns of ERK activity, we have developed a light-sensitive version of mutant MEK. This photo-switchable MEK strongly phosphorylates and activates the effector molecule of the pathway, ERK, only when exposed to 500nm light, while remaining inactive in the dark. We show that this tool can be used to induce the expected defects in tissue morphogenesis with externally applied light. Our first experiments will identify the important time windows of overactive ERK signaling during early zebrafish development that cause a defect. Ultimately, using optogenetics to probe the spatiotemporal limits of ERK signaling will shed light onto the key developmental events that determine how the early zebrafish embryo responds to abnormal signaling activity.
Understanding the evolutionary transformation of fish fins into tetrapod limbs is a fundamental problem in biology. The search for antecedents of tetrapod digits in fish has remained controversial because the distal skeletons of limbs and fins differ structurally, developmentally, and histologically. Moreover, comparisons of fins with limbs have been limited by a relative paucity of data on the cellular and molecular processes underlying the development of the fin skeleton. We provide a functional analysis, using CRISPR/Cas9 and fate mapping, of 5′ hox genes and enhancers in zebrafish that are indispensable for the development of the wrists and digits of tetrapods. We show that cells marked by the activity of an autopodial hoxa13 enhancer exclusively form elements of the fin fold, including the osteoblasts of the dermal rays. In hox13 knockout fish, we find that a marked reduction and loss of fin rays is associated with an increased number of endochondral distal radials. These discoveries reveal a cellular and genetic connection between the fin rays of fish and the digits of tetrapods and suggest that digits originated via the transition of distal cellular fates. We are currently discovering the genetic programs that have transformed fish fin rays into tetrapod limbs by using functional genomics and genetics.
O13: Molecular regulation of vascular smooth muscle cell recruitment to arteries during development

Amber N. Stratman, Timothy J. Bolan, Olivia M. Farrelly, Margaret C. Burns, Andrew E. Davis, Van N. Pham, Daniel Castranova, and Brant M. Weinstein
NICHD/NIH

The preferential recruitment of vascular smooth muscle cells (vSMCs) to arteries versus veins during early development is a well-described phenomenon that has traditionally been attributed to higher levels of blood flow rates and of shear stress through the arterial vasculature. Although the preferential recruitment of smooth muscle to arteries has been appreciated for many centuries, little is known about the molecular pathways responsible for this preference. Here, we show that the cxcl12 ligand and its receptor cxcr4 are both expressed on embryonic arteries during stages of vSMC acquisition. Using zebrafish genetic mutants, RNA/DNA over expression studies, and in vitro mechanistic analysis in primary human cell lines, we find that cxcl12/cxcr4 signaling within arterial endothelial cells leads to increased pdgf-bb ligand production, thus resulting in increased vSMC recruitment to arteries. Shortly after the onset of blood flow, expression of klf2a, a well-characterized blood flow–regulated gene that negatively regulates cxcr4 expression, transiently becomes heavily polarized to veins. This inhibits vSMC recruitment to veins by limiting expression of cxcr4 and pdgf-bb to the arterial vasculature. Together, our findings illuminate an early developmental molecular signaling axis that is regulated by blood flow and drives preferential recruitment of smooth muscle to the arterial vasculature.
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Piezo1, a mechanosensitive ion channel, is important due to its proprioceptive role and response to fluid pressure and touch. Before the discovery of Piezo1, little was known about the mechanisms behind mechanotransduction in the vertebrate specimen. The goal of this study is to characterize the expression patterns and properties of Piezo1 channels in the zebrafish retina. To investigate Piezo 1 expression in the zebrafish, protein expression levels of Piezo1 were characterized in zebrafish retina using immunoblotting and immunohistochemistry. An antibody specific for Piezo1 ion channels (Rb anti-Piezo1, Alomone Labs) was used for immunoblotting and immunohistochemical localization in the retina. In addition, various synaptic and cell type specific markers were used to determine which structures and cells were labeled with Peizo1. Specificity was determined using a preadsorptive control peptide and Piezo1 mutant zebrafish. All images were acquired using confocal microscopy and analyzed for fluorescence intensity. Preadsorption of the Peizo 1 antibody with the immunogen abolished Peizo1 immunoreactivity in the retina. Piezo1 expression was localized to cell bodies in the inner segments of cone photoreceptors and the outer plexiform layer. Strong immunoreactivity for Piezo1 was also observed in amacrine and ganglion cells in the inner retina, with the somata displaying the strongest labeling, and little or no labeling observed at either amacrine or ganglion cell processes. The expression profile suggests that a yet undiscovered role of mechanosensitive channels may exist in inner and outer retinal neurons. Our findings support the hypothesis that mechanosensitive channels, like Piezo1, could serve a critical role in regulating pressure induced mechanosensitivity in the retina. In particular, Piezo1 may be involved in regulating apoptotic influx of Ca2+ in a disease like glaucoma. Taken together, these findings support a potential role for piezo1 channels in mediating Ca2+ mediated apoptosis at the onset of elevated intraocular pressure in glaucoma.
Light-induced models of retinal degeneration are used to recapitulate the retinal pathology of age-related macular degeneration (AMD) and have been successful in non-pigmented animals. Current light-induced damage models of AMD are preformed using a prolonged dark adaptation period of seven to fourteen days followed by several days of exposure to high intensity light. The purpose of this study was to see if retinal damage would occur in pigmented zebrafish using a short period of dark adaptation followed by 48 hours of exposure to high intensity light. Pigmented wild type zebrafish were dark adapted for 24 hours and then exposed to two 8000 lumen LED lights, which were placed 30 cm away from the tank. Fish were collected at 0 hours and 48 hours post dark adaptation. A subset of fish were allowed to recuperate for 28 days before they were collected. Confocal microscopy was performed on vertical retinal sections in order to visualize cones, rods, and the outer nuclear layer of the retina. The photoreceptors in the retinas of zebrafish collected at 48 hours showed evidence of damage after exposure to continuous light in comparison to the retinas of zebrafish collected at 0 hours. Zpr-1 labeled cones and rhodopsin labeled outer segments in rods were severely truncated after light irradiation. However, photoreceptors from zebrafish that were allowed to recuperate for 28 days, still showed some signs of damage. Retinal damage in pigmented zebrafish will still occur after light irradiation with short periods of dark adaptation. The short duration dark adaptation prior to high intensity illumination provides a significant advantage of time and avoids issues related to feeding and intermittent light exposure. This modified model may be used as an alternative to light-induced models of AMD with extended periods of dark adaptation, and save valuable time and effort.
Transport and localization of cargo along the axon is essential for nervous system function. Mitochondria are a cargo of particular interest, as they have functions that are critical to neuronal health: supplying and storing ATP, maintaining calcium homeostasis, and regulating axonal branching. Perhaps not surprisingly, defective mitochondrial transport in neurons is linked to neurological disorders, such as Parkinson’s disease, Alzheimer’s, Amyotrophic Lateral Schlerosis, Hereditary Splastic Paraplegia, and many more. The mechanism governing organelle motor attachment for intracellular transport, however, is still largely unknown. Previously, we discovered that a loss-of-function mutation in Actr10, part of the dynein-dynactin complex, causes mitochondria to accumulate in distal axon terminals due to inhibition of retrograde movement. The defect in these mutants suggests a necessary interaction between dynein, Actr10, and mitochondria in order to drive retrograde transport of the organelle. However, Actr10 has no domains predicted to interact directly with mitochondria, which implies the existence of additional members of this retrograde cargo complex. To identify proteins that potentially mediate mitochondria-dynein attachment, we used immunoprecipitation and mass spectrometry to identify Actr10 interactors. Subsequently, using reverse genetics and CRISPR-Cas9 mutagenesis, we have generated mutant lines for these candidate genes to analyze the role of these proteins in mitochondrial retrograde transport. Together, this analysis will further develop our understanding of the necessary components and interactions involved in mitochondria motility along axons, a process that is vital to the overall function and health of the nervous system.
P4: An epigenetic mechanism for cavefish eye degeneration

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Coding and non-coding mutations in DNA contribute significantly to phenotypic variability during evolution. However, less is known about the role of epigenetics in this process. Although previous studies have identified eye development genes associated with the loss of eyes phenotype in the Pachón blind cave morph of the Mexican tetra Astyanax mexicanus, no inactivating mutations have been found in any of these genes. Here we show that excess DNA methylation-based epigenetic silencing promotes eye degeneration in blind cave Astyanax mexicanus. By performing parallel analyses in Astyanax mexicanus cave and surface morphs and in the zebrafish Danio rerio, we have discovered that DNA methylation mediates eye-specific gene repression and globally regulates early eye development. The most significantly hypermethylated and down-regulated genes in the cave morph are also linked to human eye disorders, suggesting the function of these genes is conserved across the vertebrates. Our results show that changes in DNA methylation-based gene repression can serve as an important molecular mechanism generating phenotypic diversity during development and evolution.
Idiopathic Scoliosis (IS), a disease that causes chronic pain, is characterized by 3D spinal curvatures with severe curves requiring surgery. IS onsets in adolescence and curves are more prevalent in girls than boys. Zebrafish (Danio rerio) have recently emerged as excellent models of IS. Our lab found that defects in motile cilia – an organelle projecting from the cell surface into extracellular space that beats back and forth to generate fluid flow – and cerebrospinal fluid (CSF)-flow cause spinal curves. However, we do not know which ciliated cells are involved nor do we know how CSF flow is sensed in the spine. My work tests the hypothesis that CSF-contacting neurons sense flow using ciliary-localized Polycystin proteins (Pkd2l1 and Pkd1l2a). To test this, I am assessing spinal curve development in mutants in these genes. I have found mild spinal curves in pkd2l1 mutants. By contrast, lethality in pkd1l2a homozygous mutants has thus far prevented robust analysis of IS-like phenotypes. I have also used cell-specific ablation techniques to determine which cell types in the zebrafish brain and spine are required for spinal linearity. To date, I have broadly ablated radial glia and neurons and am observing expected embryonic defects including twitching, early spinal curvature, paralysis, and pericardial edema. Ablations at later time points will assess the requirement of these cell types in spinal linearity. By conclusion, I hope to improve our knowledge of the role of cilia in scoliosis, knowledge which can be applied to refine treatments for those afflicted with IS.
Expression of transgene cassettes in zebrafish allows for the acute manipulation of biological systems \textit{in vivo}. Some degree of spatial control over transgene expression can be achieved using a native promoter or a Gal4-UAS driver, however this expression alone is often too broad to interrogate specific regions of interest, particularly in the brain. A combinatorial approach with a convertible cassette utilizing the cre-lox recombinase system increases specificity of transgene expression. However, the genetic intersection approach is limited by the number of distinct and characterized recombinase lines. To meet that need, we generated thirty novel cre-recombinase lines and imaged the transgene expression. These lines were characterized by registering to a common reference and imported into the Zebrafish Brain Browser. For a portion of these lines, the constituent neurotransmitter identity was predicted using the browser and confirmed by crossing the cre-recombinase line with convertible neurotransmitter reporter lines and imaging the progeny. Our work allows for increased specificity of transgene expression for functional interrogation of defined brain regions.
P7: Apical cell-cell adhesions reconcile symmetry and asymmetry in zebrafish neurulation

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The symmetric tissues and body plans of animals are paradoxically constructed with asymmetric cells. To understand how the yin-yang duality of symmetry and asymmetry are reconciled, we asked whether apical polarity proteins orchestrate the development of the mirror-symmetric zebrafish neural tube by hierarchically modulating apical cell-cell adhesions. We found that apical polarity proteins localize by a pioneer-intermediate-terminal order. Pioneer proteins establish the mirror symmetry of the neural rod by initiating two distinct types of apical adhesions: The parallel apical adhesions (PAAs) cohere cells of parallel orientation, and the novel opposing apical adhesions (OAAs) cohere cells of opposing orientation. Subsequently, intermediate proteins selectively augment the PAAs when the OAAs dissolve by endocytosis. Finally, terminal proteins are required to inflate the neural tube by generating osmotic pressure. Our findings suggest a general mechanism to construct mirror symmetric tissues: Tissue symmetry can be established by organizing asymmetric cells opposingly via adhesions.
MicroRNAs (miRNAs) are important post-transcriptional regulators that control precise gene expression program during development. It is estimated that about 30-60% of protein-coding genes harbor miRNA target sites indicating the extensive contribution of miRNAs in health and disease. Many miRNAs have distinct expression patterns and tissue-specific miRNA-mediated regulation is crucial for organogenesis. Our knowledge of lymphatic-specific miRNAs is still very limited and miRNA function in lymphatics is still largely unexplored. In this study, we used small RNA sequencing to identify miRNAs enriched in lymphatic endothelial cells and focused on characterizing the role of an evolutionarily conserved miRNA that is highly abundant in human and zebrafish lymphatic endothelial cells. We examined the role of this miRNA during lymphatic development using morpholino and CRISPR/Cas9-mediated mutagenesis in zebrafish, to take advantage of high-resolution live imaging of developing lymphatic vessels and genetic and experimental manipulation of lymphatic function. We found that the activity of this miRNA is essential for proper lymphatic vessel formation and loss-of-function causes defective lymphatic network formation. Our findings and further studies on the targets of this miRNA will add important insights into our understanding of lymphangiogenesis and lymphatic-associated diseases.
Amyotrophic Lateral Sclerosis (ALS) is a fatal, progressive neuromuscular disease. Mutations of the superoxide dismutase 1 gene (SOD1) are one known familial cause of ALS. Animal models are used to study specific neuromuscular progression of ALS since it is unsafe to regularly remove biopsies from ALS patients. Currently, Riluzole is the only FDA approved drug for the treatment of ALS with the purpose of protecting against glutamate excitotoxicity caused by SOD1 mutations. This project addresses the relative safety of early Riluzole treatment in a zebrafish model of ALS. It aims to identify anatomical and behavioral changes associated with Riluzole exposure in larval zebrafish, with specific interest on neuromuscular junction integrity in the lateral musculature and extraocular eye muscles. It also aims to characterize the side-effects of Riluzole treatment in SOD1G93R fish and support research to determine if the drug may be suitable for preventative treatment. After comparing survival between SOD1G93R, SOD1WT, and WT strains, it was found that survival between strains was significantly different (p-value < 0.001), suggesting that a stronger genetic model of ALS may be necessary to better understand the effects of drug treatment. Within each individual strain, there was no difference in the proportion of survival due to treatment conditions except within the SOD1G93R+/− line (p = 0.011). Surprisingly, compared to water, vehicle (p = 0.019) and Riluzole (p = 0.012) treatments produced significantly lower survival. Previous research has suggested that Riluzole treatment is an effective treatment by reducing cellular stress response in 4-day old zebrafish. However, these studies often disregard the behavioral consequences and complications of Riluzole while potentially overstating its benefits. This study provides novel data elucidating the effects of prolonged Riluzole exposure to provide perspective to previous and future studies of this model and disease.
P10: Establishing the larval zebrafish pectoral fin as a model for targeted axon regeneration

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The vertebrate peripheral nervous system (PNS) has significant capacity for axon regeneration. While work in many systems has addressed neuron-intrinsic factors that promote axon growth, little is known about how the regenerating growth cone interacts with cues in the environment to reinnervate target tissues with specificity. The larval zebrafish pectoral fin is an ideal model system to identify novel environmental cues required for target reinnervation due to its complex anatomy. The pectoral fin is innervated by four identified motor nerves containing dozens of axons that branch to stereotypically innervate specific regions of the fin. Three nerves, which enter the fin dorsally, and a fourth, which enters ventrally, are sorted at a plexus to innervate distinct domains of either the abductor or the adductor muscle layers. Using a laser system to transect the nerves that innervate the pectoral fin we can monitor regeneration in real time. We observe robust and specific regeneration of pectoral fin axons within two days indicating that there must be regional growth and guidance cues within the fin to guide axon growth. Easy removal of the pectoral fin allows for unbiased identification of local, injury-dependent cues in vivo in a vertebrate that are not feasible in other model systems. Here, we discuss an RNAseq approach to identify factors in the regenerating pectoral fin with expression changes after axon injury that may be required for axon growth and guidance. In future work we will use CRISPR to genetically mutate candidates to identify new factors that are required for targeted axon regeneration. Using this approach as a gateway to understand the underlying molecular-genetic mechanisms that promote sustained and directed growth of regenerating axons will generate a strong foundation for therapeutic applications aimed to promote functional PNS recovery.
X-ray microtomography (microCT) is a powerful imaging technique producing histologic resolution, 3D soft tissue reconstructions of small biological samples, such as zebrafish, which have been fixed and stained with heavy metals. To expand the capabilities of microCT to include tissue-, cell-, and protein-specific imaging, we are developing genetically-encoded tags to produce localized heavy metal contrast in whole zebrafish. One such tag, the modified soybean ascorbate peroxidase APEX2, oxidizes diaminobenzidine (DAB) to a dark brown, osmiophilic precipitate which does not readily diffuse or cross lipid membranes. APEX2 has been previously used as a genetic tag for both transmission electron (TEM) and X-ray microscopy (XRM) of tagged proteins in many contexts, including zebrafish. Because TEM and microCT staining procedures are similar, we anticipate that specific expression of APEX2 in zebrafish will show whole-body 3D localization and expression patterns of targeted tissues, cells, or proteins by microCT, even in optically opaque, whole specimens.

As a first step, we used Tol2 transgenesis to develop stable zebrafish lines expressing cytoplasmic APEX2 under the control of the ubiquitin (whole body) and HuC (neural-specific) promoters. Following fixation, DAB and osmium staining, and microCT imaging, we expect to see differential 3D expression patterns in these fish as compared to untagged controls. Furthermore, we are developing transgenic fish expressing APEX2 under control of a Gal4-responsive upstream activation sequence (UAS) to take advantage of extensive existing zebrafish libraries of tissue- and protein-specific Gal4 gene trap lines. When crossed, the Gal4 and UAS:APEX2 zebrafish will expand the number of tissue-specific microCT samples to inform diverse projects from manual and automated organ segmentation to 3D gene expression patterns and analysis of mutant phenotypes. Beyond these studies, we plan to use the CRISPR/Cas9 genome editing system to precisely fuse APEX2 with specific proteins of interest to localize and analyze these targets in 3D.
P12: Loss of NudC results in abnormal axon terminal morphology and the accumulation of vesicular cargos

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Transport of cellular cargos and organelles is essential for the formation and maintenance of neural circuits. Anterograde axonal transport (towards axon terminal) is driven by the superfamily of kinesin motor proteins. Conversely, retrograde transport (cell body directed) is driven by a single motor protein complex, cytoplasmic dynein. The mechanisms by which this one motor differentially controls retrograde transport of unique cargos is not well understood. Using forward genetics and the zebrafish posterior lateral line as a model, we identified a novel mutant strain, nudc, which has phenotypes indicative of interrupted retrograde transport. Work in fungus has demonstrated that NudC associates with the dynein complex during mitosis but a role for this protein in retrograde cargo movement has not been described. Analyses of various cargos using immunofluorescence revealed that most cargos and components of the dynein complex localize normally in nudc mutants; however, elevated levels of Cytochrome c (mitochondria protein) and p150 (dynactin component) was detected in mutant axon terminals relative to wild-type siblings. Transmission electron microscopy of mutant axon terminals revealed an accumulation of bi/multilamellar bodies filled with various tubulovesicular elements, reminiscent of enlarged autophagosomes; but no change in the number of mitochondria was observed. As Cytochrome C can also be released by mitochondria under oxidative stress, these results suggest that NudC is involved in the regulation of retrograde transport of an unidentified vesicular cargo in axon terminals, leading to mitochondrial dysfunction. Current work using live imaging of autophagosome and endosomal markers will enable us to identify the type of vesicle that accumulates in nudc mutants. Additionally, live imaging of cargo movement will reveal how loss of NudC impacts retrograde transport and leads to cargo accumulation. Ultimately, this work will provide insight into the etiology of these axonal pathologies and enhance our understanding of how Nudc regulates axonal intracellular transport.
P13: Development of the pectoral fin vasculature in zebrafish embryos

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A properly functioning circulatory system plays a critical role in human health, and understanding how blood vessels assemble is important to develop treatments for cardiovascular disease. Using super resolution microscopy, we describe the initial assembly of the vasculature supplying the pectoral fins late during the second day of zebrafish development. The pectoral fins are analogous structures to the forelimbs of mammals. The superficial location of the pectoral fin and its developing vessels make it ideal for observing and studying the events of vascular development including sprouting, anastomosis, lumenization, and the cellular rearrangements associated with these processes. The formation of the pectoral artery occurs via a stereotyped process that involves abrupt linkage to the axial vasculature to initiate blood flow throughout the vessel. This attachment leads to very rapid changes in pectoral artery morphology compared to analogous processes occurring in the intersegmental vessels of the trunk, for example. We will present some of our latest image data illuminating the mechanisms of pectoral artery growth and tubulogenesis.
Neural circuits are built through long distance connections between neurons in the central and peripheral nervous systems. The integrity of these structures depends on the transport of components between the neuronal cell body and distant axon terminals. One organelle of critical importance is mitochondria. Mitochondria perform intricate functions necessary for axonal survival, including: ATP production, calcium buffering and regulated production of reactive oxygen species. Consequently, abnormalities in axonal mitochondrial transport are associated with neurodegenerative diseases. Despite its obvious importance, the mechanisms regulating mitochondrial movement and the impact of disrupting its transport are still largely unknown. Our previous work identified Actr10 as an essential component of the retrograde mitochondrial transport machinery. Loss of Actr10 leads to accumulation of mitochondria in axon terminals due to loss of dynein-mitochondrial interaction and subsequent inhibition of retrograde mitochondrial movement. Investigation into partner proteins necessary for this interaction confirmed a physical interaction between the mitochondrial-associated protein Drp1 and Actr10. Using genetics, biochemistry and in vivo imaging, we have defined the role of Drp1 function in mitochondrial distribution. While Drp1 actively regulates mitochondrial localization in axons, phosphorylation of this GTPase at sites previously shown to be required for translocation to mitochondria, does not change mitochondrial distribution in neurons. The nature of Drp1-Actr10 interaction in mitochondrial transport regulation is still under investigation. Additionally, we have explored the functional ramifications of disrupted retrograde mitochondrial transport with Actr10 loss of function: Restriction of mitochondrial movement from axon terminals leads to elevated mitochondrial stress and decreased per mitochondria ATP production in axon terminals. Future work will explore the basic biology of mitochondrial distribution and re-distribution in axons and the connection between mitochondrial movement and known disease mechanisms.
There is high mortality during the early life stages of many fish species. This mortality comes from three sources: starvation, predation, and transport out of a favorable nursery area. The ability of larvae to overcome this mortality is key to survival and study of factors that affect mortality is ecologically and commercially significant. If food is not available during this early stage, larvae will reach a point at which they are no longer able to feed even when food becomes available, known as the Point of No Return (PNR). Past studies on PNR have been used to predict fish yields in commercial hatcheries, and are conducted on commercially valuable fish. Zebrafish are a more ecologically relevant model organism. By delaying the onset of feeding in zebrafish, we can determine their PNR. Preliminary results indicate that the PNR in zebrafish is at 8 days post fertilization. Future experiments will use the PNR to determine the mechanism of learning of feeding behavior in zebrafish.
Motivated states allow plasticity of an animal's behavior, facilitating adaptive responses to fluctuating internal homeostatic states and external challenges. A nearly universal motivated drive exists for finding resources. Despite the importance of these goal-directed behaviors for survival, the underlying neural mechanisms are still poorly understood. Here we report the identification of a novel light-search state in larval zebrafish that includes an individual preference for left or right-ward movement. After loss of illumination larvae first show movement patterns consistent with a local light-search that gradually transitions to an outward search strategy. Each phase of the search strategy allowed efficient navigation to light sources. Using mutants we identified circuitry critical for the temporal transition between local and outward search. During local search, individuals show a robust left-ward or right-ward turn bias. Using enhancer trap lines we performed a genetic ablation screen and whole brain activity mapping to identify the putative neural locus for maintaining the locomotor bias.
Fluorescent granular perithelial cells (FGPs) have been described in zebrafish as a perivascular cell type in the brain meninges which expresses lymphatic markers. While there is little known about the role these cells play in development and maintenance of the blood brain barrier, it has been observed that they exhibit distinct and replicable migratory patterns during early stages of development. Newly born FGPs emerge from the endothelium of the optic Choroidal Vascular Plexus and migrate dorsally along the developing blood vessels. This migration results in FGPs populating the perivascular space of the most external vessels of the brain, having its highest density on the optic tectum and the hindbrain. RNA-seq and single cell sequencing data has shown that FGPs demonstrate high expression of the chemokine ligands cxcl12a and cxcl12b as well as high expression of the corresponding receptors cxcr4a and cxcr4b. In zebrafish, these same specific ligand-receptor pairs have already been found to be critical in initiating and guiding the development of the trunk lymphatic network. Since FGPs express lymphatic markers and upregulation of these chemokine pairs, we suggest that these chemokines may influence FGP development and migration patterns. Through imaging and analyzing fish with cxcr4a and cxcl12b inactivating alleles, we have compiled preliminary data showing that the knockout of this receptor or ligand alone is not enough to disrupt FGP-association with the vessels nor its migratory pattern. Our future work will address the development and migration of FGPs in cxcr4b/cxcl12a single and double mutants, as well as combinations of receptor and/or ligand double mutants of these genes.
We are seeking to establish best practices for our core facility to generate and identify zebrafish with precise genome edits using CRISPR/Cas9-based methods. Because precise genome edits are generated at relatively low rates, genomes carrying precise mutations must be identified from a pool in which wild-type genomes, standardly mutagenized genomes (due to NHEJ errors) and genomes with imprecise incorporations of template are more highly represented. This low rate and diversity of outcomes presents challenges in (1) the analysis of injected F₀ embryos to determine whether a given strategy shows promise and (2) the identification of germ-line transmitting F₀ adults. We will describe the creation and detection of a targeted amino-acid substitution in the \textit{atp7a} gene using a single-stranded oligonucleotide (ss-ODN) “donor” template. To facilitate detection of precise edits, we modified our standard fluorescent-PCR fragment size analysis\textsuperscript{1} to detect acquisition of two unique restriction-enzyme sites that we also included in the ss-ODN oligo. Using this method for rate estimation, we detected one in twenty F₀ embryos carrying precise edits. In a separate cohort of adult F₀s, 0 of 16 showed germ-line transmission, but fin biopsies of an additional 15 sibling F₀s identified two candidates carrying precise edits in their soma and one of these showed germ-line transmission. The \textit{atp7a} mutation we created is a cognate to a human mutation that causes distal motor neuropathy in adolescents and young adults\textsuperscript{2}. Accordingly, we will now proceed to the medical-research phase and goal of this project, which is to characterize the potential disease progression of motor neurons in zebrafish homozygous for this mutation. We will also test the applicability of our strategies to new loci by seeking to generate single amino-acid changes in other genes under investigation at NICHD.

\textsuperscript{1}Carrington et al., PMID 26253739
\textsuperscript{2}Kennerson et al., PMID:20170900
Developing zebrafish embryos are reliant on maternally-derived yolk nutrients. Stored lipids from the yolk are re-packaged into triglyceride-rich lipoproteins in the yolk syncytial layer (YSL) and secreted into the circulation for delivery to peripheral tissues. Our previous work indicates that inhibition of lipoprotein production in the embryo results in the redistribution of triglycerides into cytoplasmic lipid droplets in the YSL. The aberrant accumulation of lipid droplets blocks light transmission through the embryo, causing the yolk to appear opaque or dark. Recently, we identified a novel dark yolk zebrafish mutant, c655. RNA sequencing of mutants and siblings suggested the mutation was located on chromosome 1 and a SNP analysis indicated the presence of a deleterious missense mutation (G863V) in the microsomal triglyceride transfer protein (mtp) gene. MTP is required for production of apolipoprotein-B (ApoB) containing lipoproteins; it transfers lipids to ApoB in the ER lumen of intestinal enterocytes, liver hepatocytes and the YSL. The c655 mutation fails to complement the previously described stl (L475P) mtp mutation, strongly indicating the G863V mtp mutation is responsible for the dark phenotype. Although stl and c655 mutants both have accumulation of lipid droplets in the YSL and delayed yolk absorption, the stl phenotype is more severe, resulting in significant growth retardation and reduced survival rate. We hypothesize that the stl mutation disrupts lipoprotein production more significantly than the c655 mutation and are currently in the process of quantifying ApoB levels and lipoprotein size in both mutants. Cell-based assays will also be performed to assess how each mutation affects ApoB binding and lipid transfer activity of mtp. Understanding how these two mutations disrupt the molecular activity of mtp and their effects on levels of circulating ApoB-containing lipoproteins may aid the design of novel inhibitors of MTP as a strategy to treat hypertriglyceridemia and cardiovascular disease.
P20: Cellular and molecular mechanisms of spontaneous CNS regeneration

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In contrast to mammals, injured zebrafish Central Nervous System (CNS) axons exhibit spontaneous regenerative capacity. We established a robust assay to transect and monitor optic nerve regeneration in live post-developmental zebrafish expressing GFP in retinal ganglion cell (RGC) neurons and their axons. Using a sharpened tungsten needle to transect the nerve proximal to the optic chiasm, we observe axonal regrowth as early as 24 hours post transection (hpt), and robust regrowth of RGC axons onto the optic tectum within 96hpt. We find that optic nerve transection does not induce significant cell death or proliferation of RGC neurons, consistent with previous results of optic nerve injury in adult zebrafish. Partial optic nerve transection results in axonal regeneration to original topographic targets within the contralateral tectum, while complete transection causes axonal growth to correct target areas, both the contralateral and ipsilateral tectum. Combined these findings suggest that regenerating axons require a preexisting tract of uninjured axons to appropriately navigate towards their original tectum, but after arriving at the optic tectum, correct topographic targeting is maintained independent of pre-existing axons.

To identify genes as entry points to further understand the mechanisms underlying spontaneous optic nerve regeneration, we screened a collection of previously identified mutants. From this ongoing screen, we identified the glycosyltransferase Lh3, to be required to direct regenerative axonal growth. In lh3 mutants, RGC axons exhibit misguided axonal growth and ultimately fail reach the optic tectum. Using a conditional allele, we demonstrate that lh3 functions during the process of active optic nerve regeneration. Finally, we show that unlike in the peripheral nervous system, where lh3 promotes target selective regeneration through collagen4a5, optic nerve regeneration occurs independent of collagen4a5 function. Combined, this identifies lh3 as a novel regulator of optic nerve regeneration independent of its role in PNS regeneration.
P21: Differential activation of neurons in the larval forebrain in response to an aversive cue

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The septo-habenular pathway bridges the forebrain and midbrain monoaminergic centers. In mouse, the posterior septum (PS) of the forebrain consists of two parallel pathways with pallial and subpallial portions that project to the medial habenulae (mHb). Ablating projection neurons from the PS to the mHb reduced anxiety and fear responses. The dorsal habenulae (dHb) of zebrafish are thought to be equivalent to the mHb of rodents. An aversive stimulus, such as electric shock (25 V, 200 ms duration), causes freezing behavior in larval zebrafish. However, delayed activation of dHb neurons is correlated with the recovery of swimming behavior. We used the same shock assay to identify forebrain neurons that might regulate the dHb response to shock. We recorded calcium activity from the telencephalon. Activated neurons could be clustered into those that react briefly or that have a sustained or a decreased response post-shock. Mapping of these groups to a larval zebrafish brain atlas will determine their spatial and temporal identity. For example, preliminary results indicate that the majority of immediate response neurons overlap with GABAergic neurons in the subpallium.
We have uncovered roles in vascular development for two unannotated genes identified using a novel zebrafish tool - Translating Ribosome Affinity Purification ("TRAP") RNAseq from “AngioTag” transgenic zebrafish expressing an affinity-tagged ribosomal protein specifically in endothelium. Utilization of this transgenic line and the TRAP technique allows us to isolate translating mRNAs specifically from endothelial cells in their native, undisturbed environment and accurately profile the in vivo endothelial translatome. Using this method we have uncovered vascular expression of a number of previously unannotated genes and have demonstrated vascular expression of several annotated genes that did not have previously reported vascular roles. We further examined the vascular roles for two unannotated genes displaying discrete vascular expression patterns. The first gene, 98293, is predominately expressed in the head and tail vasculature, and Cas9-CRISPR-induced mutants affect the brain vasculature of the larval fish. The second gene, 76721, is expressed exclusively in the tail vasculature of the fish and Cas9-CRISPR-induced mutants show defects in caudal vascular plexus development, a phenotype recapitulated in morpholino-injected embryos. Our findings demonstrate the power of AngioTag profiling to query endothelial-specific gene expression in vivo and identify novel vascular genes and gene functions. Continued study of these and other novel genes identified via AngioTag profiling will yield new insights into the molecular mechanisms regulating vascular development.
Ocular trauma is the most common injury of modern wartime, but there are no therapies available for promoting protection from, or reversal of, blast-related ocular injuries. To discover chemical compounds that promote protection, or stimulate repair, following ocular injury, we developed a whole-organism drug screen utilizing a zebrafish model of ocular trauma. To identify small molecules capable of reducing ocular trauma in zebrafish, more than 300,000 larvae were evaluated in a screen of ~3,000 human-approved drugs (six concentrations per drug and a sample size of twelve per condition). 206 existing drugs were identified that at least partially protected from ocular trauma. 50 of the best performing drugs were chosen for a series of seven orthogonal validation tests, and 13 were confirmed. Of these, five were selected as good candidates for drug optimization and further testing in mouse models. To date, three have been successfully encapsulated for long-term release, and we are evaluating these optimized lead candidates in a tertiary screen in mice using an established optic nerve crush assay. Future studies will address the cellular and molecular mechanisms of action of hit compounds for their potential use in clinical practice.
P24: Slow Myosin Heavy Chain 1 (Smyhc1) is essential for sarcomere organization in slow muscles

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Myosin II, also known as conventional myosin, is a family of motor proteins best known for their roles in muscle contraction and cytokinesis. Myosin II contains two myosin heavy chains and four myosin light chains. The myosin heavy chains are organized into three structurally and functionally distinct domains, namely the head, neck and tail domains. The globular force-producing head domain contains actin- and ATP-binding sites. The α-helical neck domain adjacent to the head domain is involved in association with the light chains. The long coiled-coil tail domain joins the myosin molecules together, forming the thick filaments of the sarcomere. Several members of the myosin gene family have been identified that exhibited different patterns of expression in slow and fast muscles of zebrafish embryos. Their gene specific functions, however, have not been well characterized. Slow muscle myosin heavy chain 1 (Smyhc1) is specifically expressed in slow muscles of zebrafish embryos and larvae. To uncover the gene function of Smyhc1, we knocked out the smyhc1 gene in zebrafish using CRISPR. Three mutant alleles were generated with reading frame shift mutations. Whole mount in situ hybridization showed the nonsense RNA decay of smyhc1 mutant transcripts in all three alleles. Loss of smyhc1 had no effect on initial development of slow muscles as indicated by the normal pattern of MyoD and Troponin C expression in slow muscle cells of mutant embryos. However, immunostaining revealed a complete disruption of myofibril organization specifically in slow, but not fast muscle fibers. The homozygous mutants were viable and able to grow into reproductive adults. However, the homozygous mutants showed a lower survival rate compared with WT and heterozygous sibling. Together, our studies indicate that Smyhc1 is indispensable for sarcomere organization in embryonic slow muscles. Ongoing research is focused on the Smyhc1 function in fish muscle growth and survival.
P25: Characterizing the function of RHOA in regulating vascular integrity

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The small, monomeric GTPase RHOA acts as a molecular switch, transducing stimuli from hormones, growth factors, cytokines, and transmembrane signaling proteins to downstream effectors of cellular signaling by phosphorylating its direct targets. Previous studies suggest that RHOA regulates many critical aspects of vascular endothelial cell biology, including focal adhesion and stress fiber formation. However, most of the functional characterization of RHOA has been performed in cell culture by overexpressing dominant negative or constitutively active forms of RHOA, or by treating endothelial cells in vitro with exogenous factors that modulate their growth and development. The in vivo functions of RHOA in regulating blood vessel integrity and development are almost completely uncharacterized. We identified a mutant in a zebrafish ortholog of RHOA (rhoaa) with severe cranial vascular integrity defects in a forward-genetic screen for dominant hemorrhage mutants. These "Bloody Mary" mutants develop extensive intracranial hemorrhage due to vascular rupture, although overall blood vessel growth and patterning appears normal. Our results suggest that cranial vascular integrity in developing zebrafish is highly sensitive to either decreased or increased rhoaa gene dosage. To gain a better understanding of the molecular mechanisms and downstream effectors of RHOA activity, we are carrying out combined in vivo/in vitro analyses of RHOA gain- and loss-of-function in the vascular endothelium, and are using phosphoproteomic profiling, experimental manipulation, and high-resolution optical imaging of vessels in living zebrafish embryos and human endothelial cells in culture. This project will enable us to identify and characterize members of the vascular RHOA signaling network, and elucidate novel potential targets for stroke prevention and treatment.
Basic research studying retinal regeneration in zebrafish (Danio rerio) is important in efforts to inform the development of ocular treatments and therapies for humans. Downregulation of the TGFβ pathway, which normally inhibits proliferation in the retina, allows Müller glia (MG) to regenerate neurons after retinal lesions. MG cells dedifferentiate, reenter the cell-cycle, and divide asymmetrically to generate a neural progenitor; then they re-differentiate into MG cells. We are generating gfap:zFUCCI (zebrafish fluorescent ubiquitination-based cell-cycle indicator) transgenic fish to study cell-cycle progression in this process with Q5 HiFi DNA Assembly or subcloning methods. Tagged sequences mAG_GEM and mCherry_Cdt1 were added to a plasmid containing the glial fibrillary acidic protein (gfap) promoter to fluoresce green in the S/G2/M phase and red in the G1 phase, respectively. MG are the only cells that express gfap in the retina. Studying the gfap:zFUCCI retina at different time points during development and after retinal lesion when manipulating cell signaling will allow for further characterization of MG cells during regeneration and development.
MAB21L2 is a highly conserved but enigmatic protein of unknown function. Human patients with mutations in MAB21L2 display eye defects, including micro- or anophthalmia and colobomas. We utilize mab21l2 mutant zebrafish as a model to identify the molecular bases of these defects. mab21l2−/− zebrafish, like the human patients, display microphthalmia and colobomas. mab21l2−/− lenses also display defects, either failing initiation entirely, or initiating on a delay and developing more slowly. There is mildly elevated cell death in the lens, possibly contributing to slower lens growth, and significant cell death in the optic stalk region, possibly contributing to coloboma. Additionally, the basement membrane between the two sides of the choroid fissure, while degraded by 48hpf in wildtype siblings, persists through 4dpf in mutants. Thus, the cause of the coloboma may be twofold- firstly, early cell death in the developing optic stalk may disrupt morphogenesis required to bring the opposing sides of the fissure together; and secondly, when the two sides do come into proximity, some crucial factor required for breakdown of the basement membrane is disrupted. In vivo time-lapse confocal imaging of mutant and wildtype embryos during optic cup morphogenesis is in progress to determine if this process is affected. mab21l2−/− lenses, when present, have patterning and differentiation defects; mutant lenses do not properly restrict foxe3 to the lens epithelium by 30hpf. crystallin alpha-a, normally present in early fibers by 30hpf, is not expressed in mab21l2−/− lenses at that timepoint, further supporting a defect in differentiation. By 3dpf, mutant lenses degrade central nuclei like wildtype lenses do, but remain much smaller. The nuclei of mab21l2−/− lens epithelial cells also appear morphologically abnormal, failing to elongate and stack into layers at the posterior of the lens. More work is underway to further characterize mab21l2−/− phenotype and probe the underlying molecular defects responsible.
P28: Exosome regulation is critical in muscle maintenance

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The goal of this study is to research the role exosomes, or extracellular vesicles, have in an in vivo model. Exosomes are evolutionary conserved from bacteria through multicellular organisms. Studies have shown exosomes play a role in cell-to-cell communication and appear to be a promising new approach for a cell-free alternative in regenerating damaged tissues. However, all studies analyzing the production, trafficking and utilization of exosomes have been in vitro. In yeast studies, vid24 was thoroughly characterized and found to play a crucial role in internalizing and degrading exosomes. When expression of this gene was induced, it resulted in an increase in exosome degradation. The homologue of this gene in fish is GID4. To create a mutant line defective in exosome trafficking, a CRISPR-cas9 construct was designed with a sgRNA to target and knock out GID4. Injected embryos that survived to adulthood were out-crossed with wild type to preserve a heterozygous line as homozygous mutations in this gene proved to be fatal in larval stages. They were then sequenced to confirm our targeted region. Sequencing showed that the fish that were able to survive to adulthood had point mutations in this region, one being Q31P. Primary characterization of these homozygous mutants showed impairment in muscle functions, and large edema around the heart. Upon closer observation and studies muscle fibers were disorganized and lacked ability to repair themselves after an injury. To prove that this is a direct cause from defective exosome uptake and degradation, functional vid24 exosomes will be injected into mutants in aim to see a rescue response. Our proposed reason for this occurrence is that when the ability to uptake and degrade exosomes is impaired, new proteins can’t be formed and distributed to the necessary target cells.
The Bone Morphogenetic Protein (BMP) pathway patterns dorsal-ventral (DV) tissues during gastrulation. 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 then phosphorylate Smad proteins, which regulate gene expression. This, 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. In the zebrafish embryo, Bmp2/7 heterodimers are the only ligands that signal in DV patterning. This arises from the heterodimer, unique ability to integrate both type-I receptors into the BMP receptor complex, as Bmpr1 preferentially binds the Bmp2 ligand, and Acvr1l exclusively binds Bmp7. I hypothesize that Bmpr1 and Acvr1l have distinct functional roles. I am performing a series of domain swap experiments to determine the components required for each receptor's specific function. We do not currently know the contribution of the two BMP type-II receptor classes, Bmpr2 and Acvr2, to the signaling complex. I am creating zebrafish mutants null for each type-II receptor class using CRISPR technology, to determine whether both classes have independent, necessary signaling functions in DV patterning.
Learning is a complex neural function that most organisms can carry out to varying degrees, yet not much is known about how this process occurs on a molecular level. Habituation is a simple form of learning in which an organism decreases or stops its response to a repeated stimulus. When presented with a repeated acoustic stimulus, larval zebrafish (*Danio rerio*) have the ability to habituate and stop responding. A mutant, *ignorance is bliss*<sup>0162</sup>, isolated through a forward genetic screen has the inability to habituate to such stimuli. A mutation in the *ap2s1* gene, which codes for the sigma subunit of the AP2 adaptor complex and plays a key role in clathrin-mediated endocytosis, was identified as the cause of the learning deficit in mutant fish. In order to understand where *ap2s1* regulates learning, we used transgenic lines for cell and tissue specific rescue in an attempt to determine which subsets of neurons require *ap2s1* expression in order to rescue wild-type behavior.
P31: MicroCT Based Quantification of the Zebrafish Gut and Brain Sub-Regions Phenotypic Characterization using Cross-Atlas Registration

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Insight from studies of wild-type organismal morphology and its comparison to abnormal samples at the levels of cell and tissue organizational levels benefit from quantitative characterization. Thus, model organism atlases ideally report physical dimensions and volumes for anatomic and cellular structures. However, classic histology-based atlases require the physical slicing through samples, a consistent plane of section, and the ability of the cutting edge to successfully cross through a tissue of interest. Failure to properly cut the sample on the first attempt may result in a slide that does not capture the desired properties of the tissue, with no capability to retry the same cut. To showcase how isotropic soft-tissue micro-computed tomography (microCT) can address these issues, we segmented the gastrointestinal tract of a 33-day post-fertilization (dpf) wild type zebrafish as a volume in its anatomic context. The isotropic nature of our soft-tissue microCT data enables digital re-slicing at any plane of section. This enables traditional histological analysis of convoluted tubular structures such as intestine without sample destruction. Virtual slicing can also be used to create both cross-sections and longitudinal sections from any tortuous structure. To address the problem of categorizing numerous, large 3D datasets derived from high throughput whole-organism scanning, we utilized manual segmentation to fine-tune our detection of wild type zebrafish brains and subregions, replacing the bulk of detection with registration algorithms to achieve higher throughput. This allowed us to quickly and semi-automatically segment multiple scans of developing zebrafish at sub-micron resolution. We identify and report volumes and volume variability across brain regions in multiple wild-type 5-dpf zebrafish as the beginnings of a foundation for quantifying normal brain tissue characteristics for phenotyping in large scale genetic alterations and drug screens.
DNA methylation is a key epigenetic mechanisms that plays crucial roles in cell type specific gene expression and cellular differentiation. DNA methyl “marks” are generated and removed by DNA methyltransferases (Dnmts) and demethylases (Tet proteins), respectively. Previous biochemical studies have shown that transcription factors and other epigenetic regulators physically interact with Dnmts and Tets and to regulate their activity and their target specificity. Genetic screens carried out in Drosophila and mouse have identified a few interacting proteins that regulate cell-type specific DNA methylation patterns, but the molecular mechanisms involved in the generation of tissue specific DNA methylation patterns are still largely unknown. We have developed a novel zebrafish transgenic reporter line that allows us to monitor dynamic changes in DNA methylation-based epigenetic regulation in intact animals during development. Using this transgenic line, we are performing the first large-scale F3 genetic screen in a vertebrate to identify recessive mutants in regulators of DNA methylation-based epigenetic gene silencing or activation. A pilot screen of twenty-five F2 families has already yielded seven mutants defective in ubiquitous (2 mutants) or tissue-specific (5 mutants) epigenetic gene silencing or activation, and we are in the process of identifying the defective genes in these mutants by RNAseq-based mapping. Identification and functional characterization of the mutated genes from our ongoing genetic screen is likely to yield many important and valuable insights into epigenetic regulation in vertebrates, just as comparable powerful genetic screens carried out in invertebrates have done.
We propose the use of high resolution imaging of millimeter scale, whole-organism sentinel species for environmental monitoring. Existing imaging methodologies do not provide the combination of resolution, field of view, and 3-dimensionality (3D) that are required for volumetric, quantitative analysis of cells and tissues in any whole organisms. Soft tissue micron-scale computed tomography (microCT), or histo-tomography is capable of generating images that provide histopathological insight for 3D phenotyping and modeling. Based upon work with larval and juvenile zebrafish, we envision the application of this new form of imaging will make it possible to establish whole-organism evaluation of aquatic meso-organisms (defined here as organisms in the 0.5 to 10 mm size range) to rapidly assess the health of aquatic ecosystems through characterizing the pathological changes across all cell and tissue types. Daphnia has been used for decades as a model organism for standard toxicity testing. Toxicological reactions, such as body length, brood size, mobility and dose-response data of daphnia to environmental pollutants are well-characterized but there is no comprehensive daphnia reference atlas. Here, we present our preliminary results of 3D reconstructions of wildtype daphnia generated from microCT images that illustrate microscopic anatomical structures. These high-resolution 3D reconstructions allow the visualization of full-volume anatomical structures comparable to histology slides, and enable rendering and visualization at different orientations, angles and slice thickness, which are not possible in histology. Renderings and segmentation can be customized to specific features of your interest. The anticipated digital images and analytics will allow the creating of a novel, web-based foundation for digital toxicological pathology reference that enables automated species identification and phenotyping for the evaluation of toxicity.

We acknowledge the Gittlen Laboratories for Cancer Research and Department of Pathology for support and funding. This project is funded, under a grant with the Pennsylvania Department of Health using Tobacco CURE Funds for Human Health and Environment. The Pennsylvania Department of Health specifically disclaims responsibility for any analysis, interpretations or conclusions.
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<td>Lightning talk</td>
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